



*OFFICERS OF THE FOURTH INTERNATIONAL  
CONGRESSES ON TROPICAL MEDICINE AND MA  
LARIA—Seated (left to right) Laurel Sawyer Schuele  
Van Hoof Stanley Missiroli Rodham, Correll Watson,  
Solkey, Gabaldon*

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Fourth International Congresses  
on Tropical Medicine  
and Malaria*

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WILBUR A. SAWYER, *Secretary General*

CAROLYN WHITLOCK, *Editor*

GERTRUDE HENDERSON, *Assistant Editor*

## Foreword

THE MEETING of the Fourth International Congresses on Tropical Medicine and Malaria was the first to be held in the Western Hemisphere. As such, it provided the initial opportunity for many workers in the western world to become personally acquainted with their colleagues from other parts of the globe. Every effort was made to arrange a meritorious scientific program and the reporters were selected because of their special knowledge of the topics assigned to them. These proceedings indicate the breadth of the program and the diversity of its subjects. However, they do not encompass the intangible but none the less substantial values provided by the Congresses, such as the spirit of fellowship and good will which pervaded all of its activities. The splendid support of the many countries in sending representatives was very gratifying. This widespread representation offered a further indication that science and health have no boundary lines and thus afford a common meeting ground for all peoples and all nations striving for the betterment of mankind. In behalf of the Organizing Committee and the Officers of the Congresses, I wish to express sincere appreciation to all delegates and members whose presence and participation contributed so materially to the success of the gathering.

*Leonard A. Scheele*

*President of the Congresses*

WASHINGTON, D. C.

*June 14, 1948*





## Introduction

A NOTABLE CONGRESS of scientists and administrators interested in tropical medicine has come to an end. The participants have returned to their many countries on all the continents to put into practice for the benefit of their peoples the new knowledge and improved methods gained from one another. But the maximum good from this international gathering will not be secured unless the scientific contributions and the results of the discussions are made widely available. By this means to accelerate progress throughout the world in the prevention and treatment of tropical diseases is the purpose of these two volumes of *Proceedings*.

The Fourth International Congresses on Tropical Medicine and Malaria were really the second joint meeting of the two pre-existing international organizations and the fourth meeting of each. Moreover, they were the first meetings of either in the Western Hemisphere. The First International Congress of Tropical Medicine met in London, August 7-12, 1913, and the Second in Cairo, December 15-22, 1928. The Third was held conjointly with the Third International Congress on Malaria in Amsterdam, September 24-October 1, 1938, as the Third International Congress on Tropical Medicine and Malaria. The First International Congress of Malaria met in Rome, October 4-6, 1925, and the Second in Algiers, May 19-24, 1930.

It was originally intended that the Fourth Congresses should be held five years after the meetings in Amsterdam in 1938, but the World War interfered and made it necessary to double the interval. When at last a meeting seemed possible the inviting government and cooperating agencies and societies encountered difficulties in tying the proposed Fourth Congresses properly into the established series. Apparently no Interim Committee had been appointed for the International Congress on Tropical Medicine, and of the thirteen members of the Interim Committee for the Congress on Malaria six had died and another could not be reached by correspondence. The consent of the known surviving members was obtained for the plan to hold the meetings in Washington, and Professor N. H. Swellengrebel, President of the Third International Congress on Malaria and member of the Interim Committee, was extremely helpful in regularizing and arranging the Fourth Congresses. The six members of the

Interim Committee with whom there was correspondence are G A Alfaro, G Pittaluga, Ed Sergent, G Bastianelli, W A P Schueffner, and N H Swellengrebel

The decade which had elapsed since the Amsterdam meeting and the many scientific discoveries of recent years accentuated the need for the Fourth Congresses and added greatly to the opportunity for making available a large accumulation of useful knowledge and at the same time stimulating international cooperation in the health field. Opportunities were opening for making disease control in the tropics more effective and less expensive. New insecticides and new drugs needed more accurate evaluation and wider application. More over the scientists and administrators in the tropical medicine field were craving an opportunity to reestablish their contacts so as to plan more effective work in their own countries and better cooperation in an international drive on tropical disease. With accelerated dissemination of new knowledge through the Congresses and strengthened central leadership through the World Health Organization and the Pan American Sanitary Bureau, there should be abundant opportunity to control disease wherever it is now entrenched.

As Secretary General I wish to express my great appreciation of the unfailing support which the Department of State has given to the Congresses through its Division of International Conferences and I wish also to thank the officers of the Congresses, and the many members of the committees and secretariat who worked untiringly to bring success. Thanks are also due to those who provided the scientific and technical exhibits and to the Sustaining Members who contributed so generously through the Intersociety Committee toward certain essential expenses. We must also express our gratitude to the Governments which sent Delegations and to the Delegates and Members who presented the valuable and timely scientific contributions which make up the greater part of these *Proceedings*.

WILBUR A. SAWYER,  
*Secretary General*

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*PARTICIPANTS IN OPENING PLENARY SESSION*  
*Seated (left to right) The Honorable George C Marshall*  
*Secretary of State, Dr Leonard A Scheele President of*  
*the Congresses Prof N H Suellengrebel Netherlands*  
*Delegate Standing Dr Willard H Wright and Dr Wil-*  
*bur A Sawyer*

# Opening Plenary Session

THE OPENING PLENARY session of the Fourth International Congresses on Tropical Medicine and Malaria was held in the Departmental Auditorium, Washington, at 11 a m, May 11, 1948. While the audience was gathering, the United States Marine Band played orchestral music.

The meeting was called to order by the Secretary General, Dr. Wilbur A. Sawyer, who stated that it was the generally accepted practice for the Chief of State of the host government to appoint a temporary president for an international conference. Accordingly, President Truman had designated Leonard A. Scheele, Surgeon General of the United States Public Health Service, as the Temporary President of the Congresses.

Dr. Scheele took the chair and spoke as follows:

The Fourth International Congresses on Tropical Medicine and Malaria are hereby convened to discuss the problems of tropical medicine and to honor the Secretary General.

## ADDRESS OF WELCOME BY THE HONORABLE GEORGE C. MARSHALL, SECRETARY OF STATE

Members of the Fourth International Congresses on Tropical Medicine and Malaria, and Guests: On behalf of the Government and the people of the United States, I welcome this distinguished gathering of scientists, physicians, and public health officials to Washington. We are honored to be host to your joint Congresses, and the Department of State, along with other Government agencies and professional societies, is happy to sponsor your sessions.

Since your last meeting at Amsterdam in 1938, the world has passed through a terrible ordeal which threatened to cancel out the progress mankind had slowly and painfully achieved through centuries of sacrifice and toil. By a supreme effort civilization was saved and in the process new discoveries and inventions were added to the store of man's accumulated knowledge. The human coco has been given another opportunity to develop an enlightened and enduring world order. The vigorous reassertion of man's constructive talents, as exemplified by this gathering of delegates from 41 countries, is reassuring to our hopes for the future.

The concentration of some of the best minds and most zealous spirits of many lands on common objectives in these conferences is convincing evidence that our world is not a conglomeration of geographic entities but a vast neighborhood of peoples. We can fly around the world now in less time than is required for the incubation

of most diseases. In the modern world isolation in the medical sense is as impossible as political and economic isolation. There is no way we can escape the consequences of each other's mischief or misfortune. There is no acceptable alternative to learning to live together in harmony and well being.

The professions you represent are in the forefront of this great humane endeavor. Statesmen and men of affairs usually and unfortunately must deal with urgent, immediate problems—the effects, and not the causes, of the discords that mar human relationships. Seldom are we able to get at the remedy for the mass misery that develops discontent, misunderstanding, and violence. That is your particular province in which you labor as benefactors of mankind.

It would be a great gain if all the prosperous and the well fed realized as well as you do that the overwhelming majority of the plain people of the earth are still primarily and necessarily con-

The conquest of diseases which hold millions weak and inefficient, the maximum production of foodstuffs on lands now yielding little are tremendously important requirements of the world situation. The tropical regions, in large measure, hold the key to both these necessary advances. They produce large quantities of materials required by the industrial areas of the temperate zones, but the potentials of the tropics largely remain to be developed. The tropical countries do import industrial products, but that market is only a fraction of what it might be.

The tropics are the habitation of perhaps half the human race, but a large portion of these people lack greatly in the advantages of modern civilization. A chief factor in restricting improvement in these respects is tropical disease. Little imagination is required to visualize the great increase in the production of food and raw materials, the stimulus to world trade, and above all the improvement in living conditions, with consequent social and cultural advances, that would result from the conquest of tropical diseases.

This situation presents a challenge that, like the Equator, cuts across national boundaries and local interests. It is an international problem and it should be solved by a pooling of the genius and the resources of many nations. That it is not insoluble from the medical standpoint has been demonstrated by numerous projects with which you are familiar. The task of convincing the governments and

The achievements and the aims of the cooperative effort represented

This spirit of generous cooperation for the common good, I am sure, will permeate all your meetings and will assure the notable success which I and my fellow Americans wish for your joint Congresses

*The following is a stenographer's transcript of the informal and extemporaneous remarks made by Secretary Marshall upon the conclusion of his prepared address*

It is almost always necessary for me, on occasions like this, to express very carefully the views of the State Department rather than specifically and only my views. Therefore, I find it advisable to read

it is more than a duty, it is a great pleasure for me to have some

conference, and noting the  
to come up, I found—and

I state this with apologies for having the temerity even to refer to technical matters of your concern in my experience—that I have not been entirely remote from some of the things that you are working with

I recall, 46 years ago, going through a cholera epidemic. I can best describe its degree and horror by stating that in the little village where I was, of about 1,200 people, 500 died in one month. We had only one doctor and

saline injections

failure after they

saw that epidemic at close range in all of its painful and devastating effects

Our only relaxation was to cross over a little river and call on a

drill at 6 o'clock, then our bath and our breakfast, that we crossed the river and called on this citizen at about 9:30 a. m. It became too hot later in the day for calls. The daughters went through the usual routine of conversation with a little giggling and finally they sang



delightfully, one playing the harp. I assisted in the burial of all three of them that afternoon. They died of Asiatic cholera. It struck us that day and developed with that rapidity. So I know a little of cholera which flourishes in parts of the tropics today.

There were other tropical diseases with which I came in contact, notably malaria in the Philippines, in those early days. Later, I was first introduced to Temporary I learn in 1905

philippines, I was very much surprised at the public reaction. Although they had had only two deaths from yellow fever in New Orleans in the previous week, I was the only guest in the principal hotel other than the members of the medical staff—I think they were called the Marine Hospital Service in those days. I observed the excitement and public reactions. People couldn't leave the train I traveled on with

control. But, of course, that is a great story in itself.

Then as I read your pamphlet, I came to references to some of our

which they knew that menace lurked, without knowing definitely how to escape its effects. Your program, I notice, even gets down to sand flies. They used to drive me frantic when I tried to sleep on beaches at night soaking wet. You are planning to eradicate them too late for my comfort, but you still talk about them, I see.

As I began to connect up these problems with my own personal experiences, your interests became very real to me, rather than abstract. Everybody agrees we should do the things you plan for the good of the world, but people generally think of them too much in the abstract. If you have had personal contact with tropical disease, it becomes a very concrete matter. We were in grave difficulty with regard to tropical disease just as we were approaching the war and when we entered into it. Again, the question was what could be done. I have found considerations of tropical medicine affecting our planning throughout my career. I, therefore, can enter with more understanding and more actual cooperative spirit than would ordinarily be the case.

Thank you very much

---

Dr. Scheele thanked the Secretary of State for his address and then introduced Dr. N. H. Swellengrebel, Chairman of the Netherlands Delegation and the President of the preceding Third International Malaria Congress.

RESPONSE TO THE ADDRESS OF WELCOME,  
 PROF. N. H. SWELLENGREBEL, NETHERLANDS

Mr. Chairman, Mr. Secretary, on behalf of all the delegates and

men and women of many nationalities and from numerous countries all over the world. Obviously, I cannot know this. As a matter of fact, I only know what is in my own mind. I shall assume, now, that my mind does not greatly differ from the average mind of this gathering. Starting from that one point, I may venture to say that I

1939, shortly after the beginning of the war. I had been reading Lincoln's two addresses, sculptured on the walls of the memorial. The Gettysburg Address is the best known, but at that time the second one appealed to me most. And yesterday, Lincoln's words recurred to me, his well known words which run as follows: "With malice towards none, with charity for all, let us drive on to finish the work we are in." It struck me at that time that these words could be taken as an appropriate epigram for these Congresses, an epitome of what is deeply hidden in our minds. Now, somebody may indignantly exclaim,

mention the remarkable achievements obtained of which we are going

is quite true that we shall hear of all these new achievements better and more fully than I could expose them here. It is exactly for that reason that I believe it worth while to halt for a moment; yea, even to lose sight for a moment of all these most interesting topics and to ask ourselves what are we here for; what does the United States Government, which has invited us here, ultimately expect from us? Are we here to read papers and have them printed? In that case, sending them to the usual periodicals for publication would give them even wider circulation. No! What we are here for primarily is reconstruction, to tie again the bonds of international relationships, personal friendship, and scientific cooperation which the war has severed.

In a short address at the official dinner of the Congress of Microbiology in New York in September 1939, I expressed a fear that the war would mean isolation, especially for the small neutral countries of Western Europe hemmed in by the great contending powers. At that time I did not realize how terribly true was that remark of mine, how completely we were to be shut off from the life giving West

t. Even the  
that reason  
long forced

eastward, snapped its bond and turned west to the exclusion of all other things, "Westward Ho!"—but now in a very different sense from that in which it was used formerly—"Westward Ho" became the mainspring of our life.

And now those who have invited us to this Congress turn our minds in another direction. Perhaps unconsciously they put us on the shoulder as if they were saying to us, "No, my friend, you are wrong. Your mind has been warped by years of suffering. There is no East and no West, but only the brotherhood of science devoted to the well being of the human race. Forget your geographical predilection and simply apply yourself to the work in hand." And by this generous admonition the critic I quoted just now may seem to have been confirmed in his criticism, still, I hope that this admonition has not put me wholly wrong. However that may be, everyone surely will concur in repeating the sentence with which my address commenced, expressing our heartfelt gratitude for the cordial words of welcome extended to us on this day.

---

The chairman, Dr. Scheele, then suggested that it would be desirable to introduce the chairmen of the Government delegations and asked each one to rise when Dr. Sawyer, the Secretary General, called his name. As the roll was called the chairman of each delegation stood and was applauded. The President then brought up various items of business, beginning with the adoption of the rules of procedure. The proposed rules were in Document No. 5, copies of which had been distributed to all the governments at the time invitations were issued. The delegates and members had copies in English, French, or Spanish at the time of registration. There seemed therefore, to be no need for an extended discussion and in the absence of objections, the President declared the rules of procedure approved.

Under the provisions of article 10 (a) of the rules of procedure,

:

serve as secretary of this committee, and he had his arrangements for the committee to meet briefly in room B immediately after the

## ELECTION OF OFFICERS

The chairman called attention to article 5 of the rules of procedure providing that the Congresses should elect a Permanent President and three Vice Presidents. Article 14 specified that on questions of organization each country participating should have one vote only. The Chair proposed first to call for nominations and then to have the

and Temporary President of the Congresses, as President.

As the nomination was seconded and there were no further nominations, the Secretary General, Dr. Sawyer, temporarily took the chair and invited the chairmen of delegations to vote on the nomination. After the show of hands Dr. Sawyer announced that the Temporary President, Dr. Leonard A. Scheele, had been elected President of the Congresses.

## REMARKS BY THE PRESIDENT, DR. LEONARD A. SCHEELE

Thank you very much. I deem it an especial privilege to have been

office of Surgeon General of the United States Public Health Service.

Secretary Marshall referred to the size of the world—how the world has shrunk now with air transport and the movement of people from place to place. This meeting I think is especially historic, coming as it does at the end of World War II which was more of a global war than any other we have ever had. We hope there will be no wars in the future, but if there are we can be sure that they will be even more global than was the last war. We can be sure that almost every citizen of the world will be involved in any wars of the future.

were developed before the war and put into fairly extensive use during the war, are now being used much more extensively and do, in fact, offer such great promise that we may in time see some of the diseases which are insect borne wiped out through these more effective control measures.

War also placed stress on the need for research. Unfortunately, in times of war much research is directed to the applied fields, away from fundamental fields and one of the things that we are happy to see now is the great building up again of fundamental and basic research along with developmental and applied research. We have made some strides—grand strides, to be sure—in chemotherapy. We

hold that the future will hold even greater advances in this field and even greater hope for cure of many of our tropical diseases

We have seen something else come about in the United States, and other countries too, in recent years. We have seen the banding

hope to do our small share in the study of tropical diseases. We hope to begin breaking ground within the next 6 months for a large clinical and laboratory research center in Bethesda to supplement the present buildings of the National Institute of Health and in that building we will carry forward more research than ever in the past in the field of tropical diseases

to make my small contribution by presiding at this and later plenary sessions and, of course, we hope that there will be many, many more successful Congresses following this one

I do not wish to take more of your time when you have an excellent scientific program beginning this afternoon, so we will proceed with the next order of business

The meeting then proceeded to elect three Vice Presidents, following the same procedure as in the previous election

Gen Marcel Vaucel of France, Sir Sahib Sokhey of India, Sir Gordon Covell of the United Kingdom, Prof I Van Hoof of Belgium and Dr Mustapha Bey Fahmy Sorour of Egypt were placed in nomination

The President then called the names of the five candidates. Each head of a government delegation was asked to vote for three of the candidates by raising his hand. The following votes were received by General sor Van Hoof, who were thus

In accord with article 7 of the rules of procedure, the meeting then elected three Honorary Presidents and six Honorary Vice Presidents on the nomination of the Organizing Committee. The nominations were presented by Dr Sawyer, Secretary of the Organizing Committee, and were as follows

As Honorary Presidents Dr Richard P Strong of the United States, Prof Jerome Rodham of Belgium, and Sir Malcolm Watson of the United Kingdom

As Honorary Vice Presidents Col Charles F Craig of the United States, Dr Edmond Sergent of France, Prof Alberto Missiroli of Italy, Sir Sahib Singh Sokhey of India, Dr C K Chu of China, and Dr Arnoldo Gabaldon of Venezuela

The heads of government delegations voted in favor of these

## APPOINTMENT OF COMMITTEES

The President appointed a Committee on Resolutions composed of the following members Dr Ernest Carroll Faust of the United States, chairman, Gen Maurice Peltier of French West Africa, Dr Louis Van den Berghe of Belgium, Lt Col M K Afridi of Pakistan, Dr Francisco J Dy of the Republic of the Philippines, Dr Mustapha Bey Sorour of Egypt, and Dr Heitor P Froes of Brazil Mr William L Breese would serve as secretary

As it had been the custom for the International Congresses to

H Wright as representative of the Organizing Committée, and the Secretary General The chairman or secretary of this committee would submit the nominations for members of the Interim Committee at the closing plenary session on Tuesday, May 18

The President then called on the Secretary General, Dr Sawyer, who introduced the section conveners and also the two principal officers of the secretariat, Dr Willard H Wright, Assistant Secretary General for Program, and Mr Harold G Kissick, Assistant Secretary General for Administration

After announcing the principal events of the afternoon, particularly the official reception at the Pan American Union, and thanking the United States Marine Band for the musical program, the President declared the meeting adjourned

## OFFICIAL RECEPTION

The Assistant Secretary of State, the Honorable Willard L. Thorp, and Mrs. Thorp, acted as hosts for the United States Government at a reception for the delegates, members, associates, and guests of the Fourth International Congresses on Tropical Medicine and Malaria at the Pan American Union and its Aztec Garden. Between 5 and 7 o'clock in the afternoon over 1,200 persons became better acquainted on this first day of the Congresses, and enjoyed refreshments and the

... .. were Dr. Leonard Scheele, and Dr. Wilbur A. Sawyer, secretary general, and Mrs. Sawyer

## THE BANQUET

... ..  
... ..  
the number of 448, gathered to converse, dine, and enjoy orchestral music and after dinner speeches. They were eager to hear the announcement of the award of the Laveran Prize and the Walter Reed Medal for distinguished achievements in tropical medicine, and to have a part in honoring the recipients.

The toastmaster was Dr. Leonard A. Scheele, president of the Congresses. As the first of the after dinner speakers he introduced Dr. Lewis W. Hackett from Buenos Aires, well known as a malarialogist on the staff of the International Health Division of the Rockefeller Foundation. Other speakers who followed were Prof. Jerome Rod

... ..  
... ..  
the United States of America bestowed the Walter Reed Medal on Dr. Swellengrebel. Those speeches for which manuscripts were made available are printed below.

### ADDRESS BY DR. LEWIS W. HACKETT

A memorable meeting is drawing to a close and we are all about to separate on our centrifugal paths except those who must pass the summer in Washington, who have the sympathy of those of us who

are escaping to the Tropics I can't tell you how happy I am to be permitted to address, without restriction of subject or fear of warning bell a gathering which probably contains more people with a common interest in the diseases of warm climates than were ever assembled before. The armies of the plasmodia, trichophytons, leishmania trypanosomes, and rickettsias are peering out at us with apprehension from behind their collapsing bulwarks of Cancer and Capricorn.

I may have been selected for this distinguished honor because I happen to know all the malarialogists in the world over 50 years of age, and now there are rumors going around that no more are to be turned out. These rumors have been spread, I am sure, by the tropical

imbricated ringworm. None of these is very mysterious and they are all peculiarly intractable a combination of characters rather depressing to the workers in this field. So, they have rather aggressively adopted all the exotic diseases as well, such as leprosy, Rocky Mountain spotted fever, Chagas disease, plague, cholera and the rest, which has made it unnecessary for many of them to go to the tropics or simi-

brilliant and unpronounceable addition to the list. I suppose it will be called for short, Shortt's Disease. And finally, as you all know, one day last week they absorbed malaria as well and we shall have only one Congress hereafter. All I care to say at this time is that the vote was taken under the most suspicious circumstances by our chairman, General Covell, who asked for a show of hands in the dark and then forgot to call for the "noes." Tropical medicine seems to have taken over malaria just as it is about to disappear, like the famous personage who was appointed royal dentist to King Ladislao of Poland on the day His Majesty lost his last tooth.

What the tropical medicine men have always admired about the malarialogists, I think, is their extreme mobility. We malarialogists early took to heart the dictum of Paracelsus that a physician does not learn by sitting at home behind the other places to see what is being organized to pay his expenses in this respect, having visited not only all the malarious areas of the globe, but also many interesting nonmalarious places besides, in order to find out why there was no malaria there. I have passed many extenu



ating days on the French Riviera with its enigmatic problems, and was once lost for a week in the Black Forest, there was one staggering period at Pilsen and an expedition I shall never forget to the center of the island of Capri which few specialists in other tropical diseases have ever managed to visit when on service

Some of us who are present have attended all four International

only experimental trials in the laboratory I took a couple of pounds with me to Italy when I first went there in 1924 and Missiroli and I sneaked over to Sardinia to try it out by ourselves This was the subject of the first paper I ever wrote, and it was delivered before the First International Congress on Malaria We were all excited because, if I may be permitted to mix a metaphor, mosquito control was then a virgin field, pregnant with possibilities

The Rockefeller Foundation in collaboration with the Italian Government had set up the Malaria Experiment Station in a lovely little palace called the Farnesina, rented to us for 1 lira a year by the city of Rome Professor Missiroli was director and Drs Raffaele and LaFace were on the staff This little palace had some interesting frescoes on the walls and ceilings and we had to admit the public on one day each month A tourist once asked our stupid but obliging porter Ferdinando whether we had any frescoes by Raffaele, the famous medieval Italian painter Now of course we didn't have any of Raphael's frescoes but Ferdinando said to the delighted tourist "It may be, I don't know, but Raffaele himself is upstairs"

The Second Congress on Malaria was held in Algiers in 1930 and ushered in the larvicide era, a method strongly supported by the Americans, and particularly by the Rockefeller Foundation When I landed in Algiers all the public buildings were decorated with flags bearing the letters "R F" Thomson, the British protozoologist, had about convinced me that they stood for Rockefeller Foundation when we discovered that it was a national holiday and R F might have stood for Republique Francaise This was the meeting at which it was finally admitted by all except a few die hards and the quinine trust that drugs were not a preventive measure in malaria We were limited at that time to quinine and methylene blue and their effect in reducing the transmission of malaria was practically nil We larviciders were pre

to collaborate immediately :  
ebbing tide

called us the mud hen school of malarialogists who insisted, like the Baptists, in getting into the water all over Professor Missiroli used to baptize young malarialogists who visited our work in Sardinia by leading them across a wide and shallow stream to see something on

the other side Those who remained behind lost his confidence for ever and Missiroli never believed in any results they might subsequently report, even in the field of chemotherapy

At the Third Congress in Amsterdam in 1938 we had refined our techniques and the theme was species sanitation We had learned that not only the European *maculipennis* but many other widespread species were really complexes and could often be attacked piecemeal By that time Professor Swellengrebel had already started the attack on the adult mosquito in houses and stables with insecticide sprays containing pyrethrum He found that by adding oil of sassafras to the mixture he could excite the mosquitoes resting in dark corners and cause them to fly out into the open where they could be knocked down by the pyrethrum Unfortunately, the essential oils also excited and irritated the cattle in the barns so that the infuriated cows attacked the bulls, and the owners called a halt

And now, here we are in Washington in 1948 with the attack on the adult mosquito in progress The world is ushering in the age of

But the chemotherapists are

Very promising new drugs and embryonic chemicals are on trial I have even heard that an extraordinarily effective drug has just been discovered for which at present there is no disease Once again we are on the threshold of to be a malaria

e malarialogists

Malaria made

ral in the field

of malaria was disastrous, to be a paludologue (as the French call us)—a specialist only in swamps and marshes—was to be a failure The study of this disease in the 1920's and 1930's was at once a bond between us and a cause of bitter dissension Grassi said he once

grave and scientists made arrangements with spiritualistic mediums in order to continue the discussion if possible from another plane

Much of this conflict of ideas and personalities came to a focus in the Malaria Commission of the League of Nations The Anglo Saxons were apt to be much more abrupt than the Europeans I well re

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Boudreau, to tear his argument to pieces. A French member always referred to us indiscriminately as "les savants americains" but managed somehow to intimate that we must know a lot about some other disease since we were evidently pretty ignorant about malaria, as indeed in the beginning we were. Col James was once said to have remarked "So and so agreed with me today for the first time in 20 years, I must be wrong"

All the remarks and discussions had to be translated into another language after the speaker had sat down, and quite often of course lost most of their savor. One translator, however, was a brilliant exception. He was eloquent and at times impassioned. Some unexcitable Briton would mutter a devastating argument in an impassive monotone and this dynamic interpreter would jump to his feet, clench

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malaria. Has that a significance? One of our forward looking colleagues, well known as an eradicator of gambiae and aegypti, has openly insinuated that malarialogists today are nothing but potential malefactors. We are capable of trying to preserve some malaria somewhere in order to study it some more. In fact we seem to be hung upon a tragic dilemma. Condemned to be buried as fossils or executed as criminals. It has been gradually dawning upon me that Dr Soper has reached back into the past to exhibit me upon this platform as a specimen of the old mud hen malarialogist, excavated in a reasonably good state of preservation, except for some hairs missing from the primitive brow and occiput. I date from the palaeozoic era, somewhere between the tertiary and quaternary periods, resurrected to pronounce the obituary of malaria which is about to be executed by poison as enemy No 1 of mankind.

Dr Boyd's prodigious book which has been looming on the horizon for so long, may, it seems, reach us just in time to serve as the tomb stone of our portentous enemy. And Dr Soper may even now be preparing for his next big campaign, to eradicate malarialogists.

Well, I imagine all this is a little premature. DDT is a terrific weapon even in the hands of ignoramuses, but in the end, there is no substitute for knowledge. What will happen, it seems to me, is what

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mildly protested that the malarialogists have overemphasized the complexities and uniqueness of their disease. We all suspected that this was because he himself was occupied in emphasizing the importance of yellow fever. But the turn of events is likely I think to support

Dr Sawyer There was a time when I would have hotly contested the point, having devoted almost the whole of my life to one disease. But as David Starr Jordan said, just to see anything clearly and

jungles, and savannas which lie in and near the tropics

Mankind is in need of the tropics Paul Morand has said that the defect of this age is velocity, but more probably it is that there are too many people The tropics offer at least a temporary relief, until mankind can bring itself in a burst of Malthusianism to curb its philoprogenitiveness The tropical rain forest is still almost uninhabited

that man as an individual is a puny animal but as a species he has the force of a geological process or a climatic shift Some of us here may live to see the day when Gorgas' prediction will be substantiated, that it will eventually cost no more to keep a family healthy

adequate budgets, more  
oramuses, the ministers  
eapons, new discoveries,

and great accomplishments ere we meet again

#### REMARKS OF PROF JEROME A H RODHAIN

When a man obtains the privilege of living to an age that some people call "respectable" he gets the opportunity to remember many events, some happy ones, and many others unhappy And so it was my lot to survive two World Wars, fighting the first one and bearing during the last one the heavy load of domination of my country, Belgium, by the enemy Surely this time was the worst time of my life

But not

in Algiers, where the French celebrated Laveran's discovery of the etiological agent of malaria It was in 1930, real peacetime, and nearly all nations were represented at the Congress For those great commemoration days France appeared in the full splendor of her glory, and some of you who attended this Congress will remember that as I do

The second which I attended was held in Amsterdam in 1938 and was an important meeting, but already the political sky of Europe was darkened with black clouds. During those days the events of Munich brought some release before the tragic outbreak of the war in 1939.

And now I have the very great pleasure of attending the Fourth International Congresses on Tropical Medicine and Malaria in this magnificent city, in which we all feel beats the real heart of the people of the United States.

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gap has been for a long time a dark point in the clinical and therapeutic

vances and discoveries that have happened during the 45 years of my career since I had my first contact with tropical medicine. When I landed in Africa for the first time in 1903 we knew only one specific drug for our defense against the various tropical diseases—that was quinine. Thus I went out with quinine and a mosquito net. Amoebic dysentery and its liver complications ended often fatally. Trypanosomiasis was always a mortal infection, and schistosomiasis, leishmaniasis, kala azar, and filariasis could not be cured. I have seen the numerous advances which put an end to this distressing situation. Following one after the other the etiology (say transmission) of sleeping sickness, bilharzia infections, and leishmaniasis were cleared up. Chemotherapy occupied soon an important place with a series

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amoebiasis cases, and soon antimony compounds showed activity against leishmaniasis and schistosomiasis. Then in 1920 Bayer 205 appeared, and also tryparsamide, which was the first drug that could cure the late stages of sleeping sickness. And most recently there have appeared the derivatives of guanidine and amidines with their powerful protective activity and lastly the new products active against filariasis.

Against malaria we have nowadays plasmochine and atabrine reinforced with paludrine and chloroquine and many allied compounds. With them and the helpful new insecticides of the classes of DDT and gammexane we may hope for the eradication of malaria from large parts of the world.

This progress which I have remembered here represents an enormous amount of combined work by men who were altogether motivated by the will to reduce human distress. Alas, we have not the same powerful means against the moral diseases from which humanity

matter of fact excluded.

In conclusion, ladies and gentlemen, I wish to say in the name of my country and in my own how thankful we are to the Government of the United States and to the Organizing Committee of the Congresses for the friendly and hearty reception we have enjoyed. This forges a new link in the friendship between Belgium and the United States.

#### SPEECH BY DR. HECTOR P. FRÉES

Ladies and gentlemen I feel quite uncomfortable at this moment while reflecting that "speeches are just like babies, always easy to conceive . . . but sometimes difficult to deliver!"

from this distinguished audience, I would rather talk about the mutual influence, since ancient times, between malaria and poetry

As malaria has been, for centuries, a scourge to the Roman "Campagna", it is no wonder that such celebrated poets as Plautus, Terence

or that

as the

the dreadful quartana

Let us recall, also, the celebrated Italian poet D'Annunzio, who

and he tells us, according to an old Roman legend, how she changes youth into old age.

Now comes Giovanni Cena, who describes in his poem "The Mosquito" . . . that little shadow flying across the humid air and sucking the blood of the Father, and later inoculating the parasites into the children

So spoke the poets (one third doctors) most of them in an epoch when doctors didn't know much more than poets about malaria! There was also young doctor Ronald Ross (one third poet) whom

the wise and gouty Patrick Manson put on the trail of the discovery of the transmission of malaria by mosquitoes because this young fellow was going to leave for India, a place with abundance of mosquitoes and thousands of people with rigors and fever

You all know about Manson's fantastic theory which instantly became Ross' Great Problem, and you also remember how Ross proved (thanks to his sponsor) the transmission of malaria (let us be precise of avian malaria) by mosquitoes

I beg to remind you now of a poem that Ross himself composed on the day when he saw in the body of a brown female mosquito that had been fed (at his expense) by Mr Husein Khan the same spherical living bodies which he had observed before while examining blood films of such a human guinea pig

Please listen to the poet

*"This day relenting God  
Hath placed within my hand  
A wondrous thing, and God  
Be praised, at this command,  
Seeking His secret deeds  
With tears and toiling breath,  
I found thy cunning seeds  
O million-murdering death!  
I know this little thing  
A myriad men will save  
O Death, where is thy sting,  
Thy Victory, O Grave!"*

These verses by Ross, the poet, have been inscribed on one side of a monument erected in Calcutta, to commemorate the great discovery of Ross, the doctor, half a century ago. But in spite of our progress, since then (thanks to Grassi and his "zanzaroni" thanks to so many Italian, French, German, Dutch, English, American and international scientists) we now know how malaria has continued to help the Angel or Devil of Death in his job of weakening and destroying the human race

A Brazilian doctor, whom you all know, has tried to put into Portuguese verse (just to strike the attention of some idle students) the main differential characteristics between *Culex* and *Anopheles* mosquitoes, as you can appreciate from the two following quotations

*'Anopheles wings are striped,  
Her robes are brown, you'll say  
Culex wings are rather clear,  
She dresses always in gray*

*'From both you may know the larvae  
Even without microscope  
Anopheles larvae float,  
The others have a periscope!'*

Nowadays poets rather should greet DDT and praise Gummexane,  
 (and please don't take me),

to solve  
 this problem (including old and modest Alfonse Laveran), I would  
 like in conclusion, to attempt to imitate Ross (and please don't take  
 me for a snob)

*"O Death, where is thy sting?  
 Anopheles, your offspring?  
 Malariaologists, your job?"*

PRESENTATION OF THE LAVERAN PRIZE TO PROF HENRY E. SHORTT  
 BY PROF. N H SWELLENGREBEL

I have a document here which I shall read aloud, that all present  
 may know its contents

"The Permanent Committee of the International Malaria Con-

would be considered as the one of greatest importance among the

been appointed by the Permanent Committee to select the recipient  
 of the Laveran prize This Commission is composed of the follow  
 ing official delegates

Medecin Général Maurice Peltier  
 Professor J Rodhain  
 Dr Paul F Russell  
 Général Maurice Vaucl  
 N H Swellengrebel, chairman

"On the authority bestowed on the commission by the Permanent

The Commission  
 Médecin Général Maurice Peltier  
 Professor J Rodhain  
 Dr Paul F Russell  
 Général Maurice Vaucl  
 N H Swellengrebel, chairman"



Professor Shortt, by the reading of this document which I have the honor to present to you, you are officially proclaimed the recipient of the Laveran prize, 1948. Let me add a few words unofficially. Some may think that this is an honor conferred upon you by the present Congress. It is nothing of the sort. The idea that you would

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sandflies. All believed it. Nobody could prove it. You could, and you did!

And now you have proved the existence of exoerythrocytic stages in the life cycle of simian and human plasmodia. All believed in their existence. Chemotherapists acted on this belief. Among innumerable synthetic compounds, they selected those killing avian exoerythrocytic stages. Still it was a belief. You put a certainty in its stead.

You are a builder of bridges spanning chasms of ignorance. You are a constructor of foundations, like that other constructor of foundations, the man we all admire so much, Sir Rickard Christophers. I want to compare you to him. I want your name coupled with his. Some of our friends from India and Pakistan called Sir Rickard their father. In time to come they may call you by that name.

And now. Are we, this Congress, going to honor you? No sir! You are honoring us by being one of us. By awarding you this prize, we are patting ourselves on the back, and we feel what fine fellows we are by counting you as a member of our Congress.

So we extend to you our most cordial thanks for having allowed us to use you for our own egotistical and self-laudatory purposes!

Professor Shortt briefly expressed his thanks and appreciation of the honor.

#### PRESENTATION OF THE WALTER REED MEDAL TO PROF. N. H. SWELLENGREBEL BY DR. ROLLA E. DYER

Mr. Chairman, officers of the Congresses, distinguished guests, delegates, and members. It is my privilege to represent on this occasion of the other

The Walter Reed Medal was established in 1936 by the American meritorious achievement of such achievement is, and governments which have been recipients of the award. The Walter Reed Medal was first granted to the Rockefeller Foundation for its outstanding work in the investigation of yellow fever. In 1939, the medal was awarded to Dr. W. B. Castle of Harvard University, for his investigation of the

anemias of Puerto Rico In 1940, Dr Herbert C Clark received the medal for his outstanding accomplishments in the study of tropical diseases of man and animals In 1942, the medal was bestowed upon the Government of Brazil for its epoch making achievement in the elimination of *Anopheles gambiae* A year later, Dr Carlos Finlay, of Cuba, was awarded the medal posthumously for his fundamental studies in the transmission of yellow fever Brigadier General James Stevens Simmons was the recipient of the award in 1944 for his distinguished contributions in the prevention and control of tropical diseases in the United States Army overseas In 1946, the medal was given to Dr Paul Russell in recognition of his services in the control of malaria in the United States Army

various times with Professor Mesnil in Paris, in Switzerland with

Toghem In 1913 he  
th his distinguished  
Vogel At the end

Our eminent colleague is perhaps best known for his classical studies on the anopheline vectors of malaria in the Netherlands East Indies However, we must not forget that he has also made outstanding contributions in other fields of tropical medicine His early

the life cycle of the intestinal protozoa His work on plague was notable in its additions to the knowledge of the biology of the arthropod vectors of this disease and of the rodent reservoir hosts.

For many years he has devoted much of his time to the study of malaria He was a member of the justly famous Malaria Commission of the League of Nations. As such member, he has studied the disease in many parts of the world including the Far East, Southern Europe, the Americas, British India, South Africa, and other areas. In 1939, he visited Surinam at the invitation of the International Refugee Colonization Society to examine the possibilities for the colonization of refugees in this dependency of the Netherlands. The results of

these researches are published in the classical report under the title of "Health of White Settlers in Surinam."

Many honors have come to the recipient of the present award. He is an honorary member of the American Society of Tropical Medicine, honorary life member of the New York Academy of Sciences, foreign honorary member of the American Society of Parasitologists, honorary corresponding member of the South African Medical Association, and honorary member of the Société Belge de Médecine Tropicale. In 1937, he received the Bernhard Nocht Medal, in the same year the Darling prize, and in 1938 the Darling Medal.

He has also been honored by coming to the rostrum of the Division of Tropical Hygiene at the Netherlands, Knight of the Order of the Netherlands Lion, president of the Third International Tropical Medicine Congress, and President of the International Tropical Medicine Congress.

He has been particularly active in the epidemiology and control of malaria, investigations which have resulted in such outstanding benefits to mankind in many parts of the world. In behalf of the society and these Congresses may I express the hope that you may yet have before you many years of productive service in this, your chosen field.

#### RESPONSE BY PROFESSOR SWELLENGREBEL

Mr. Chairman, ladies and gentlemen. In the face of this great honor which has been conferred on me I must confess to a weakness—the weakness of vanity, which on a certain occasion made me feel disappointed. I almost had the chance to lay a wreath on General Washington's tomb—and I lost it. It was all to the good, for someone much better qualified to do so performed the ceremony. But it was a disappointment, because from my youth onward I have always been an ardent admirer of this great general and statesman, the American War of Independence I have always found one of the most fascinating subjects in history, and the glorious American Commonwealth has always had my admiring sympathy.

And now you will understand what it means to me that this people, represented by that great assembly of scientists, The American Society of Tropical Medicine, has awarded to me the Walter Reed Medal. It would have been an honor in all circumstances, but coming from the Americans, and in the city which bears my hero's name, it is an honor, indeed.

And therefore, Dr. Dyer, I beg you to allow me once more to shake you by the hand, you, American citizen, representing to me your glorious liberty bringing Nation.

## CLOSING PLENARY SESSION

The Congresses were convened by their President for the closing plenary session at 2 15 p m on Tuesday, May 18, 1948, in the Departmental Auditorium

### REPORT OF COMMITTEE ON CREDENTIALS

The report of the Committee on Credentials was read by its chairman Dr A Neghme Rodriguez, as follows

Credentials of delegates, as well as other communications transmitting the names of persons designated to represent their governments, were examined

The committee recommended that the credentials of each delegation be submitted to the Secretary General as far in advance of the opening meeting as possible, and that the chairman of each delegation be given the original credentials for presentation to the Secretary General immediately upon arrival at the site of the Congresses

The committee acknowledges with appreciation the assistance of delegates in securing all the information required for the compilation of this report

Signed by Amador Neghme, chairman, A H Baldwin and Joao Braga de Azevedo, members, and Mr J Ward Lowe, secretary

A motion to accept the report was adopted

### REPORT OF COMMITTEE ON RESOLUTIONS

The report of the Resolutions Committee was next presented by its chairman, Dr Ernest Carroll Faust. Copies of the report as signed by Dr Faust, chairman, and Mr William L Breese secretary, had been previously distributed in English French and Spanish

At the suggestion of the President the resolutions were read separately and each was voted upon before the next was taken up. The President announced that all members of the Congresses were entitled to vote on all resolutions except the first, which dealt with a question of organization. Only the chairmen of government delegations were privileged to vote on that resolution

### RESOLUTIONS OF THE CONGRESSES

*Resolution I* ESTABLISHMENT OF AN INTERNATIONAL CONGRESS ON TROPICAL MEDICINE AND MALARIA AND INTERIM COMMITTEE

WHEREAS the International Congress on Malaria, meeting as Section I of the Fourth International Congress on Tropical Medicine and

Malaria, has unanimously approved the following report of its committee composed of Dr Carlos Alvarado, Prof Giulio Raffaele and Dr N H Swellengrebel

1 At present malaria still is a disease of such outstanding importance in the tropics and subtropics that it still requires close attention With regard to international scientific congresses the importance of this subject ought to be emphasized by allotting to it a place well above that of a simple section in the program of the Congresses

2 At the same time the committee realizes that it is not desirable that there should exist an entirely independent malaria congress On the contrary, this congress should be closely and permanently joined with that on tropical medicine in general But this junction should be effected in such a fashion as not to infringe on the condition formulated in paragraph 1

3 The committee recommends that this junction be brought about in future by instituting one Congress on Tropical Medicine and Malaria under one President, assisted by two Vice Presidents, one for the Division of Tropical Medicine and one for the Division of Malaria

4 *Interim*  
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Its members will be selected with due regard to an adequate representation of both divisions Therefore

The Fourth International Congresses on Tropical Medicine and Malaria

**RESOLVE 1** That the International Congress of Tropical Medicine and the International Congress of Malaria shall be permanently merged to form the International Congress on Tropical Medicine and Malaria, and shall in the future meet as one entity under one President and two Vice Presidents, one for the Tropical Medicine Division and one for the Malaria Division of the Congress

and Malaria

3 That the interim committee be authorized to make preparations for the fifth International Congress on Tropical Medicine and Malaria and determine the time and place of the Congress in collaboration with the host government

Resolution I was voted upon by the chairmen of delegations and was unanimously adopted

*Resolution II INVITATIONS TO THE FIFTH INTERNATIONAL CONGRESS ON TROPICAL MEDICINE AND MALARIA*

The Fourth International Congresses on Tropical Medicine and Malaria

RESOLVE 1 To express their thanks to the Governments of the

tive countries

2 To request the interim committee of the Congress to consider these and any other official invitations which may be received

Resolution II was voted upon by the members of the Congresses and unanimously adopted

### *Resolution III COOPERATION WITH THE WORLD HEALTH ORGANIZATION*

The Fourth International Congresses on Tropical Medicine and Malaria

RESOLVE 1 To express their accord with the ideals, aims and pursuits of the World Health Organization, and to offer their full support to the World Health Organization in the accomplishment of its objectives

2 To express their gratification that the malaria, schistosomiasis and plague experts of the World Health Organization were present at Washington during the sessions of the Fourth Congresses, and that invitations were extended to the members of the Congresses to express their views

3 To express the hope that the World Health Organization will

present

The proposed  
read and amend  
that the word "  
olution IV, as amended and adopted, was as follows

### *Resolution IV PROGRAM OF FUTURE CONGRESSES*

The Fourth International Congresses on Tropical Medicine and Malaria

RESOLVE To request the interim committee to consider the advisability of the program of the Fifth Congress providing for contributed papers limited to one paper per author, the only other restriction

gether with the conclusions drawn therefrom

### *Resolution V INTERNATIONAL CENTERS FOR THE COORDINATION OF STUDIES OF DISEASES IN THE TROPICS*

The Fourth International Congresses on Tropical Medicine and Malaria

**RESOLVE** To call the attention of the World Health Organization to the desirability of establishing international centers for coordinating the studies of diseases in the tropics, particularly the rickettsioses, the intestinal protozoan infections and diseases due to domestic arthropods, and setting up standard procedures which can be adopted by workers in all countries

Resolution V was unanimously adopted

#### *Resolution VI EXPERT COMMITTEE ON PLAGUE*

**WHEREAS** in the light of present knowledge of the effectiveness of the newer insecticides, rodenticides, prophylactic and therapeutic measures and other methods of control, it is believed possible to eliminate plague as a human menace, therefore the Fourth International

World Health  
established to

study and plan action for the elimination of plague as a human menace

Resolution VI was unanimously adopted

#### *Resolution VII NUTRITIONAL DEFICIENCIES*

The Fourth International Congresses on Tropical Medicine and Malaria

**RESOLVE** To reemphasize the present inadequate knowledge of nutritional deficiency diseases and incomplete data concerning nutrition in many countries, particularly in the Tropics, and

peoples of various countries

Resolution VII was adopted by unanimous vote

#### *Resolution VIII CHAGAS' DISEASE AND LEISHMANIASIS*

The Fourth International Congresses on Tropical Medicine and Malaria

**RESOLVE** 1 To request the Pan American Sanitary Bureau to act as a center of information and coordination between the institutions and investigators interested in the study of Chagas' disease and leishmaniasis in order to help formulate a methodical joint investigation program in the Western Hemisphere

2 To instruct the interim committee to communicate directly or through a subcommittee with the Pan American Sanitary Bureau on this matter

Resolution VIII was unanimously adopted

*Resolution IX HEALTH EDUCATION*

The Fourth International Congresses on Tropical Medicine and Malaria

RECOMMEND That, since health education is essential to the success of public health administration in the Tropics even more than elsewhere, the most modern methods of instruction and demonstration should be applied to increase the support and participation of the public in health conservation

Resolution IX was unanimously adopted

*Resolution X TRIBUTE TO THE HOSTS AND THOSE COOPERATING IN THE CONGRESSES*

The Fourth International Congresses on Tropical Medicine and Malaria

RESOLVE 1 To express their profound gratitude to the President of the United States, the Honorable Harry S. Truman, for his invita-

ident  
, and  
gram,  
Exhibits, Entertainment, Extra Congress Activities, Finance Public Relations, Reed and Ross Celebrations and Women's Hospitality, of the Secretary General of the Congresses, Dr. Wilbur A. Sawyer, of his associates in the Secretariat, as well as the officers and personnel of the various sections, for their contribution to the success of the Congresses

3 To express their sincere thanks to the governmental agencies,

Resolution X was adopted by acclamation

Dr. Mark F. Boyd then proposed that the name of Harvard University be added to the list of institutions to which thanks is expressed. The President accepted the proposal in behalf of the meeting,

thanks  
he sug-  
gestion as the consensus of the meeting and thanked Dr. Faust, Mr. Breese, and all the members of the Resolutions Committee for their excellent work in developing the resolutions.

The Resolutions of the Congresses as adopted are presented below also in French and Spanish



## RESOLUTIONS DES CONGRÈS

## I ÉTABLISSEMENT D'UN CONGRÈS INTERNATIONAL DE MÉDECINE TROPICALE ET DE PALUDISME ET D'UN COMITÉ INTERIMAIRES

ATTENDU QUE Le Congrès International du Paludisme représen

Raffaele et du Dr N H Swellengrebel

1 Le paludisme est encore une maladie d'une telle importance dans les régions tropicales et semi tropicales qu'il convient de continuer à l'étudier avec soin En ce qui concerne les congrès scientifiques internationaux, il serait bon de souligner la portée de la question en accordant à celle-ci une place beaucoup plus importante au programme des Congrès que celle représentée par une simple section

2 Toutefois, le comité se rend également compte que l'existence d'un congrès du paludisme entièrement indépendant n'est pas à de

1 Cette jonction  
pas enfreindre la

venir  
idisme  
Vice

Présidents, dont l'un pour la Division de la Médecine Tropicale et l'autre pour la Division du Paludisme

4 Le comité recommande, pour l'avenir, la création d'un comité intérimaire pour le Congrès de Médecine Tropicale et de Paludisme, qui agira en qualité de représentant des deux Divisions, et dont les membres seront choisis de manière à accorder une représentation adéquate à chacune d'elles,

Les Quatrièmes Congrès Internationaux de Médecine Tropicale et de Paludisme

l'autre pour la Division du Paludisme

l'autre pour la Division du Paludisme

2 Qu'un Comité intérimaire, qui accordera une représentation adéquate à ces deux Divisions, sera établi pour veiller à l'exécution des décisions des Quatrièmes Congrès Internationaux de Médecine Tropicale et de Paludisme

3 Que le Comité intérimaire sera autorisé à procéder à l'organisation du 5ème Congrès International de Médecine Tropicale et de Paludisme

Paludisme et a en fixer la date et le lieu en collaboration avec le gouvernement invitant

## II INVITATIONS AU CINQUIEME CONGRES INTERNATIONAL DE MEDECINE TROPICALE ET DE PALUDISME

Les Quatriemes Congres Internationaux de Medecine Tropicale et de Paludisme,

DECIDENT 1 D'exprimer leurs remerciements aux Gouvernements

2 De charger le Comité intermaire du Congres d'examiner ces invitations et toutes autres invitations officielles qui pourraient être reçues

## III COOPERATION AVEC L'ORGANISATION MONDIALE DE LA SANTE

Les Quatriemes Congres Internationaux de Médecine Tropicale et de Paludisme

DECIDENT 1 D'exprimer leur adhésion aux idéals, aux buts et aux travaux de l'Organisation Mondiale de la Santé et de lui offrir leur plein appui dans la réalisation de ses desseins

2 D'exprimer le plaisir que leur a cause la présence à Washington des experts de cette Organisation en matière de Schistosomiase, Peste et de Paludisme, ainsi que l'invitation qui a été faite aux membres des

procédera à l'organisation du 5eme Congrès International de Médecine Tropicale et de Paludisme

## IV PROGRAMME DES CONGRES ULTERIEURS

Les Quatriemes Congres Internationaux de Médecine Tropicale et de Paludisme,

DECIDENT De charger le Comité intermaire d'examiner l'opportunité du programme du 5eme Congrès, qui prévoit la libre soumission de communications à raison d'une communication par auteur, la seule restriction stipulée portant sur leur longueur et sur la date de leur

tionnées d'un compte rendu extensif des discussions et des conclusions auxquelles elles ont abouti

## V CENTRES INTERNATIONAUX POUR LA COORDINATION DE L'ÉTUDE DES MALADIES DANS LES TROPIQUES

Les Quatrièmes Congrès Internationaux de Médecine Tropicale et de Paludisme,

DECIDENT D'attirer l'attention de l'Organisation Mondiale de la Santé sur l'opportunité de créer des centres internationaux pour coordonner l'étude des maladies dans les tropiques particulièrement des maladies rickettsiennes, des infections intestinales protozoaires et des maladies dues aux arthropodes domestiques, et d'établir des méthodes standard de travail pouvant être adoptées dans tous les pays

## VI LE COMITÉ D'EXPERTS SUR LA PESTE

CONSIDÉRANT À la lumière des connaissances actuelles sur l'efficacité des récents insecticides et rodenticides des mesures prophylactiques et thérapeutiques, et des autres méthodes de contrôle, il est permis de croire que la menace que représente la peste pour l'humanité sera éliminée, en conséquence

Les Quatrièmes Congrès Internationaux sur la Médecine Tropicale et de Paludisme,

DECIDENT Que ces Congrès recommandent à l'Organisation Mondiale de la Santé qu'un comité d'experts sur la Peste soit créé pour étudier et établir la méthode à suivre pour éliminer la menace que représente la Peste pour l'humanité

## VII DEFICIENCES ALIMENTAIRES

Les Quatrièmes Congrès Internationaux de Médecine Tropicale et de Paludisme

DECIDENT De souligner à nouveau l'insuffisance des connaissances actuelles en ce qui concerne les maladies dues aux déficiences alimentaires et le manque de données complètes en matière de nutrition dans

sations intéressées prennent des dispositions appropriées pour favoriser les recherches cliniques sur les déficiences alimentaires humaines dans l'objet de relever le niveau de nutrition des peuples de divers pays

## VIII MALADIE DE CHAGAS ET LEISHMANIOSE

Les Quatrièmes Congrès Internationaux de Médecine Tropicale et de Paludisme

DECIDENT 1 De prier le Bureau Sanitaire Panaméricain de bien vouloir servir de centre d'information et de coordination entre les institutions et les chercheurs qui s'intéressent à l'étude de la maladie de Chagas et de la leishmaniose afin d'aider à formuler un programme

de recherches à poursuivre méthodiquement et en commun dans l'Hémisphère Occidental

2 De charger le Comité intérimaire d'entrer en communication avec le Bureau Sanitaire Panaméricain à ce sujet, soit directement, soit par l'intermédiaire d'un sous-comité

## IX EDUCATION SANITAIRE

Les Quatrièmes Congrès Internationaux de Médecine Tropicale et de Paludisme,

Considérant que l'éducation en matière d'hygiène est essentielle au succès de l'administration de la santé publique, dans les tropiques plus encore qu'ailleurs,

RECOMMANDENT Que les méthodes d'instruction et de démonstration les plus modernes soient employées pour éveiller l'intérêt et encourager la participation du public en ce qui concerne la conservation de la santé

## X. Vœu de Remerciements

Les Quatrièmes Congrès Internationaux de Médecine Tropicale et de Paludisme,

DESIRENT 1 Exprimer leur profonde gratitude au Président des Etats Unis, l'Honorable Harry S. Truman, pour la convocation et

au Président des Congrès, le  
l'Organisation et de liaison,

Bureau et au personnel des diverses Sections, dont la diligence et les efforts ont contribué au succès des Congrès.

3 Exprimer leurs sincères remerciements aux organismes gouvernementaux et privés, à l'Union Panaméricaine, au Bureau Sanitaire Panaméricain, de même qu'aux Membres souscripteurs et à toutes autres personnes qui ont apporté aux Congrès un concours précieux

## RESOLUCIONES DE LOS CONGRESOS

### I ESTABLECIMIENTO DE UN CONGRESO INTERNACIONAL DE MEDICINA TROPICAL Y PALUDISMO Y DE UN COMITÉ PROVISIONAL

Los Cuartos Congresos Internacionales de Medicina Tropical y Paludismo,

CONSIDERANDO Que el Congreso Internacional de Paludismo, reunido como Sección V de los Cuartos Congresos Internacionales de



## CLOSING PLENARY SESSION

sus generosas invitaciones para que el V Congreso de Mal y Paludismo se reúna en sus respectivos países  
2 Encarecer que el Comité Provisional del Congreso tanto éstas como cualesquieras otras invitaciones oficiales

### III COLABORACIÓN CON LA ORGANIZACIÓN MUNDIAL DE MEDICINA

Los Cuartos Congresos Internacionales de Medicina Paludismo,  
RESUELVA. 1 Expresar su acuerdo con los ideales, fin y propósitos de la Organización Mundial de la Salud y ofrecer completo apoyo en la prosecución de sus objetivos

2 Expresar su satisfacción porque los peritos en paludismo, en la peste de la Organización Mundial de la Salud, en Washington, a las sesiones de los Cuartos Congresos, porque se expidieron invitaciones a sus miembros para asistir a los puntos de vista

3 Expresar la esperanza de que la Organización Mundial de la Salud colabore con el Comité Provisional en los preparativos del V Congreso Internacional de Medicina Tropical y Paludismo

### IV PROGRAMA DE LOS CONGRESOS FUTUROS

Los Cuartos Congresos Internacionales de Medicina Tropical y Paludismo,  
RESUELVA. Encarecer al Comité Provisional que considere la conveniencia de que en el programa del V Congreso se mantengan los trabajos de autores no invitados a un solo trabajo por cada autor, sin o restricción que la de la extensión y la fecha de la presentación, que los trabajos seleccionados para el Congreso, que en lo referente a los trabajos seleccionados para ser leídos en el Congreso la mayor parte del tiempo se dedique a su discusión, y que el Acta Final contenga los trabajos seleccionados una extensión de las deliberaciones y las conclusiones que de ellas se deriven

### V. CENTROS INTERNACIONALES PARA LA COORDINACIÓN DE ESTUDIOS DE LAS ENFERMEDADES TROPICALES

Los Cuartos Congresos Internacionales de Medicina Tropical y Paludismo,  
RESUELVA. Llamar la atención de la Organización Mundial de la Salud sobre la conveniencia de establecer centros internacionales para la coordinación de estudios de enfermedades de los trópicos, especialmente la rickettsiasis, las infecciones intestinales de protozoos y en enfermedades causadas por los artrópodos domésticos, y la adopción de métodos uniformes que puedan ser seguidos por los intereses de todos los países.

## VI COMITE DE PERITOS EN PESTE

Los Cuartos Congresos Internacionales de Medicina Tropical y Paludismo,

CONSIDERANDO Que en vista del conocimiento actual de la eficacia de los nuevos insecticidas, rodenticidas, medidas profilácticas y otros medios de combatir enfermedades se cree posible la extinción de la peste como azote de la humanidad,

RESUELVE Que los presentes Congresos recomienden a la Organización Mundial de la Salud la creación de un comité de peritos en la peste para estudiar un plan de acción que conduzca a eliminarla como azote de la humanidad

## VII DEFICIENCIAS DE LA NUTRICIÓN

Los Cuartos Congresos Internacionales de Medicina Tropical y Paludismo,

RESUELVEN Poner nuevamente de manifiesto el escaso conocimiento actual de las enfermedades originadas por deficiencias de la nutrición y lo incompleto de los informes que se tienen respecto a la nutrición

... y Agricultor  
... organismos in  
interesados en los pasos necesarios para ampliar los estudios clínicos sobre las deficiencias de la nutrición en el ser humano a fin de mejorar la alimentación de los pueblos de diversos países

## VIII ENFERMEDAD DE CHAGAS Y LEISHMANIOSIS

Los Cuartos Congresos Internacionales de Medicina Tropical y Paludismo,

RESUELVE  
constituya  
tuciones e

de un subcomité, se comuniquen con la Oficina Sanitaria Panamericana en relación con este asunto

## IX EDUCACION EN MATERIAS DE SANIDAD

Los Cuartos Congresos Internacionales de Medicina Tropical y Paludismo,

RECOMIENDAN Que siendo la educación en materias de sanidad esencial para el buen éxito en la administración de servicios públicos de sanidad en los tropicos, más que en ninguna otra parte, deben implantarse los sistemas mas modernos de instrucción y demostración para estimular el apoyo y participación del público en la preservación de la salud

# **X. RECONOCIMIENTO A LOS ESTADOS UNIDOS Y A LAS ENTIDADES QUE COLABORARON EN LOS CONGRESOS**

Los Cuartos Congresos Internacionales de Medicina Tropical y Paludismo,

**RESUELVEN:** 1 Expresar su profunda gratitud al Hon Harry S Truman, Presidente de los Estados Unidos, por su iniciativa al convocar y preparar la presente reunión

2 Dejar constancia de su reconocimiento por la labor del Presidente de los Congresos, Dr. Leonard A. Scheele; la del Comité Organizador y el Comité de Enlace entre Sociedades; la de los Comités del Programa de Beltsville, Exhibiciones, Agasajos, Actividades Extraordinarias, Finanzas, Relaciones Públicas, Commemoraciones de Reed y Ross y Femenino de Hospitalidad; la del Secretario General, Dr Wilbur A. Sawyer; la de sus asociados en la Secretaría, y la de los funcionarios y el personal de las distintas Secciones por su aportación al buen

Panamericana, los miembros contribuyentes y a cuantos prestaron su valiosa cooperación.

The President of the Congresses, as chairman of the closing plenary session, called on Dr Willard H Wright, member and secretary of the Committee on Nominations for Members of the Interim Committee for the committee's report, which he presented as follows:

## **REPORT OF THE COMMITTEE ON NOMINATIONS FOR MEMBERS OF THE INTERIM COMMITTEE**

Dr. Willard H. Wright, Secretary of the Committee on Nominations for Members of the Interim Committee, presented its report as follows:

The Committee met on May 14 with Dr. Scheele in the chair and first discussed Resolution No. 1 which you have adopted this after-

Committee voted that the Interim Committee should consist of 13 members. It was the consensus of opinion that these 13 members should be distributed geographically as follows:

Africa.....	2	Asia.....	3
North and Central America.....	2	Australia and New Zealand.....	1
South America.....	2	Europe.....	3

With the adoption of this geographical distribution, Mr President, the Committee would like to nominate the following representatives for members

Bey Fahmy Sorour (Egypt).



Asia—Dr H C Hou (China), Lt Col Jaswant Singh (India), Lt Col M K Afridi (Pakistan)

Australia and New Zealand—Prof E Ford (Australia)

America (South)—Dr H P Froes (Brazil), Dr A Gabaldon (Venezuela)

America (North and Central)—Dr E H Hinman (United States), Dr M E Bustamante (Mexico)

A motion was made, seconded and adopted that the nominations be closed. The list of nominations was then voted on as a whole and all the persons on it were elected members of the Interim Committee.

Dr Wright then presented a second part of the Report of the Committee on Nominations for Members of the Interim Committee as follows:

The Committee recommended, in case the above nominations were approved, that the officers of the Interim Committee be as follows:

Chairman Gen M Vaucl of France.

Vice Chairmen Dr H P Froes of Brazil (for Tropical Medicine), Lt Col M K Afridi of Pakistan (for Malaria)

Secretary Treasurer Dr L Van Hoof of Belgium

A motion was adopted that officers for the Interim Committee

announced that the Interim

officers designated. It was suggested that all the members of that committee who were still present meet in Room B immediately after the adjournment of the Congresses.

After pausing to permit the Secretary General to make an announcement, the President gave his Farewell Address.

#### FAREWELL ADDRESS OF THE PRESIDENT OF THE CONGRESSES DR LEONARD A SCHIELE

Delegates, members, and friends attending the Fourth International Congresses of Tropical Diseases and Malaria. The last item on the agenda is a few words by your President. You have heard me several times but I assure you that the remarks I am about to make will be brief.

The physical and mental well being of the people of all nations is the foundation of international political and economic stability.

of communicable diseases is particularly hazardous to the health of people everywhere and therefore should demand our immediate attention. The need for international cooperation in preventing their spread has been intensified not only by the population shifts of World War II, which occasioned the greatest movement of people the world has ever known but also by the increased speed of travel. Recent technical developments in transportation have shortened round the world

travel time to less than the incubation period of most diseases. Certain exotic tropical diseases have already penetrated the quarantine barriers of some lands. No nation is self-sufficient in protecting itself from them. The best defense is attack, and attack at the source is the

cooperation instilled into them by their code of ethics and by their professional esprit de corps. Among scientists who deal with tropical medicine, in particular, a method of cooperation has evolved which is a working reality in current international affairs as is shown by the success of these Congresses. Governments have formed continental and intercontinental and world wide organizations designed to raise the public health status of their citizens to higher levels than any nation individually could aspire to attain.

One of the events of the last war was the recognition of the significance of the work of a number of fundamental workers over a period of several decades—that of Einstein, Fermi, Bohr, Rutherford, Meitner, and others. A group of American scientists with almost unlimited resources took the fundamental discoveries of these many men

Our appreciation of speed of travel has made us conscious of the smallness of the world. The enrollment of 1,200 people in these Congresses is further evidence of the awareness of the peoples of all the world of the importance of progress in medicine and particularly in tropical diseases, including malaria, and of their willingness to meet to discuss common problems.

Last night at dinner, Dr. Hackett carried us back over the progress made at the preceding three Congresses. You have all seen the

pose in banding together two groups, one on tropical diseases and the other on malaria, into a single organization with unity of purpose.

The Interim Commission, which you have now created to consider matters which are of help to the Congresses, has the right and the

lands for the Fifth Congress. Thus, the wheels of progress roll on.

Finally, I wish to say in behalf of my colleagues from the United

We have tried to have a good program for you. You have expressed yourselves as having enjoyed the meetings; we thank you for that.

Soon we shall disperse. We hope that some of you will stay behind for a while at least to travel through this country and visit some of the laboratories and some of your friends whom you have heard here and others who were unable to come.

We wish to thank you for coming to attend the

With ;  
cine and Malaria are adjourned

## SPECIAL EXERCISES

### COMMEMORATION OF DEMONSTRATION BY WALTER REED OF MOSQUITO TRANSMISSION OF YELLOW FEVER

The Departmental Auditorium was the scene of a special meeting to commemorate the demonstration by Walter Reed of the mosquito transmission of yellow fever. On the platform, behind the speakers and distinguished guests, were the massed flags of the many nations

Band

Bliss,

Surgeon General of the United States Army, who introduced the Chairman, Dr. Fred L. Soper, Director of the Pan American Sanitary Bureau. The chairman made a brief address and then introduced the distinguished guests who were seated on the platform and in the audience in the front rows of seats. Among them were persons associated with the yellow fever experiments in Cuba, including one of the volunteer subjects.

After a musical selection by the United States Army Band, the chairman gave a résumé of the developments in the fight against yellow fever since the demonstration of the method of its transmission. He then introduced the orator of the evening, Dr. Philip S. Hench, who gave an address on Walter Reed and the Conquest of Yellow Fever. Many historical pictures were thrown on the screen to illustrate this address.

ing pages.

### OPENING REMARKS BY MAJ GEN RAYMOND W. BLISS, SURGEON GENERAL, UNITED STATES ARMY

Distinguished Colleagues, Ladies, and Gentlemen: We are come here this evening to honor a man whose contributions to world health and medical science are universally revered. Walter Reed's memory is especially dear to the Army Medical Department. I am proud to

men on the one hand and the support, faith, and understanding on the other brought to the world one of the great achievements in science. Their example has made the medical problems during and after two World Wars seem less formidable and has spurred us on in our efforts to solve them.

Other speakers will recall for you the story of Walter Reed's work, and the parts played by his loyal assistants and the volunteers to whom the world owes so much. I wish to speak of the heritage left for us by this great scientist. It is not enough that we have named one of our general hospitals in his honor or that his likeness resides there, a perpetual reminder of his place in medicine. The real heritage lies in the spirit and in the zeal which his name and achievements inspire in the men who have followed him. It is a living force which has motivated men, in the Medical Corps and out, to strive for perfection in the scientific approach and constantly to seek the answer to the many baffling medical and scientific questions of the day.

The humanitarian aspect of the conquest of yellow fever is known to all of us. A scourge was conquered and a great burden lifted from the shoulders of the peoples of many lands. Less well understood is

the gains to be reaped in success. In this calm judgment and sterling courage lies another heritage, and a challenge.

As scientists and medical men and women from many countries we look out upon a troubled world. We cannot resolve its troubles, but we can set an example of cooperation within the bounds of our profession. Walter Reed's world

health. That you feel some measure of the same responsibility is proved by your attendance at this great International Congress. Walter Reed encouraged international cooperation in Cuba—how much greater is the need now.

It now becomes my pleasure to place this session in the able hands

methods and procedures.

As chairman, he will have something to say about yellow fever since the days of Walter Reed, and will introduce our distinguished guests and the principal speaker.

**ADDRESS OF THE CHAIRMAN AND INTRODUCTION OF DISTINGUISHED GUESTS BY DR. FRED L. SOPER, DIRECTOR OF THE PAN AMERICAN SANITARY BUREAU**

It is a pleasure to have you here, and to have that the

control of the most dreaded scourge of the American tropics. Yellow fever at one time or another has invaded every country on the Ameri

## WALTER REED COMMEMORATIVE ME

can continent, including Canada. Several of the I including England, France, Portugal, Spain, ar fered serious summer outbreaks, and a large part tribate to this disease.

Today it is recognized that jungle yellow fever and Africa, together with rapid transportation, a tial threat to Asia and the Pacific, which have nere to many areas long free of yellow fever infection harbor the mosquito vector, *Aedes aegypti*.

As the problem is an international one, so have tions to its solution. We are commemorating h and dramatic incident in the colorful history o incident which led to the first successful campaign this disease. In a few brief months, Walter Reed demonstrated through conclusive human experi *Aedes aegypti* mosquito can transmit yellow fever son under certain definite conditions. It was th which led to the organization of anti mosquito mti tol of yellow fever.

The rapid success of the Army commission wa cause of the preceding work of Dr. Carlos Finlay.

any of Medical, Physical and Natural Sciences as an outstanding example of epidemiological rea always place Finlay alongside Sir Patrick Manson ment of our knowledge of the insect transmission c

Unfortunately there has been failure on the pa to recognize the importance of the work of Finl recognize the indispensable nature of the work do This has been due, I am sure, to a failure to a difference in the approach to the problem. I thoroughly convinced himself of the function of t as the vector of yellow fever that he considered h tion unnecessary. I take pleasure in quoting at

"If these results are compared with those of the United States Army commission they certainly appear undemonstrative. This can be explained by their objective with the consequent limitation of the infective mosquitoes to one, and at most two, with the greater number of infective cases being past the third day of their illness, and to the incubation of the mosquito (extrinsic incubation) being under 12 days."

The son quotes the father

"On not a few occasions during the 20 years of our experimentation we were tempted to carry out our inoculations in such a manner as to obtain more decisive experimental results. We received communications to this effect from various persons who after having heard the explanation of our doctrine and our insufficient experimental results argued that the end justified the means, but they never could persuade us to abuse the trust deposited in us by those who had submitted to our inoculations on the ground that they were essentially inoffensive."

In the Reed experiments the danger inherent in infection with yellow fever was fully accepted. After due consideration of all the factors involved, the decision was taken to risk human life in order to learn the truth. Fortunately it was possible to secure human volunteers who readily agreed to experimentation knowing full well the risk they ran.

Ladies and Gentlemen, we are happy to have among our honored guests this evening Mr. James L. Hanberry who was one of the original volunteers for the Reed experiments. Mr. Hanberry slept in contact with clothing and victims of yellow fever ever from this prolonged exposure. He then submitted himself to an experiment in which he was bitten by mosquitoes which had previously fed on known yellow fever cases. After a short incubation period, he suffered an attack of the disease, from which he fortunately recovered.

In the absence of Mrs. Walter Reed, who is unable to be with us tonight, I take pleasure in saluting the surviving members of the family through the person of Maj. Gen. Walter Lawrence Reed, the son of Walter Reed, who was on military duty in another part of Cuba at the time his father's important work was in progress.

We are happy to have Gen. Merritt W. Ireland, former Surgeon General of the Army, who was on duty during the Reed experiments and was intensely interested in them. Now he is vice president of the Walter Reed Memorial Association.

maximum of encouragement and support. We are most happy to have General Kean with us this evening.

We are further honored this evening by the presence of Brig Gen Albert E Truby, who, as Lieutenant Truby, was commanding officer of the post hospital of Camp Columbia, which was the base of operations for the Reed experiments

We have with us this evening in the audience Miss Blossom Reed, daughter of Major Reed, whose modesty has prevented her joining us on the platform

fever Commission and the one fatality from yellow fever connected with its work

As chairman of this Commemorative Meeting, I must take the liberty of introducing from the floor Dr F F Russell, formerly of the Medical Corps of the United States Army, who played such an important part in some of the later developments in yellow fever control following up the work of the Reed group

(Interval for a musical selection by the U S Army Band.)

Before introducing the orator of the evening, I wish to give you a brief résumé of the important developments in the fight against yellow fever which have occurred since the epoch making demonstration of the Reed Commission that yellow fever is transmitted by *Aedes aegypti* mosquitoes I shall disregard entirely further laboratory developments and the work which has been done with yellow fever vaccine

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disease were undertaken in a number of countries in the Americas By 1915 the workers in yellow fever were convinced that through anti aegypti measures applied to a relatively small number of endemic centers in the Americas it should be possible to eradicate the disease completely from this hemisphere Such excellent results seemed to be obtained in this campaign of eradication that in the middle 1920's it was believed that the disease continued to exist only in a small area of northeast Brazil

The unexpected reports of the disease at isolated points in Brazil,

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to exist in South America as an animal disease of the forest entirely independent of humans and of the aegypti mosquito This jungle disease constitutes a permanent source of virus for the accidental re-infection of such communities as permit the continued existence of a heavy aegypti infestation and can be reached by modern rapid travel



"If these results are compared with those of the United States Army commission they certainly appear undemonstrative. This can be explained by their objective with the consequent limitation of the infective mosquitoes to one, and at most two, with the greater number of infective cases being past the third day of their illness, and to the incubation of the mosquito (extrinsic incubation) being under 12 days."

The son quotes the father

"On not a few occasions during the 20 years of our experimentation we were tempted to carry out our inoculations in such a manner as to obtain more decisive experimental results. We received communications to this effect from various persons who after having heard the explanation of our doctrine and our insufficient experimental results argued that the end justified the means, but they never could persuade us to abuse the trust deposited in us by those who had submitted to our inoculations on the ground that they were essentially inoffensive."

In the Reed experiments the danger inherent in infection with yellow fever was fully accepted. After due consideration of all the factors involved, the decision was taken to risk human life in order to learn the truth. Fortunately it was possible to secure human volunteers who readily agreed to experimentation knowing full well the risk they ran.

Ladies and Gentlemen, we are happy to have among our honored guests this evening Mr. James L. Hanberry who was one of the original volunteers for the Reed experiments. Mr. Hanberry slept

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posure. He then submitted himself to an experiment in which he was bitten by mosquitoes which had previously fed on known yellow fever cases. After a short incubation period, he suffered an attack of the disease, from which he fortunately recovered.

In the absence of Mrs. Walter Reed, who is unable to be with us tonight, I take pleasure in saluting the surviving members of the family through the person of Maj. Gen. Walter Lawrence Reed, the son of Walter Reed, who was on military duty in another part of Cuba at the time his father's important work was in progress.

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W. Ireland, former Surgeon  
General Ireland was on duty  
the Army during the Reed experiments and was intensely interested in them. Now he is vice president of the Walter Reed Memorial Association.

Brig. Gen. Jefferson R. Kean, a great grandson of Thomas Jefferson was Chief Surgeon of the Department of Western Cuba, which included Havana, at the time of the experiments. He gave Reed a maximum of encouragement and support. We are most happy to have General Kean with us this evening.

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In the audience we have also Admiral and Mrs James O Gawne Mrs Gawne represents the Lazear family, being a first cousin of Dr Jesse W Lazear whom you will all remember as one of the Yellow Fever Commission and the one fatality from yellow fever connected with its work

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the urban and maritime disease from man to man by the *Aedes aegypti* mosquito is not the basic cycle on which the disease depends for its continued existence in the Americas Yellow fever has been proven to exist in South America as an animal disease of the forest entirely independent of humans and of the *aegypti* mosquito This jungle disease constitutes a permanent source of virus for the accidental re-infection of such communities as permit the continued existence of a heavy *aegypti* infestation and can be reached by modern rapid travel

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WALTER REED AND THE CONQUEST OF YELLOW FEVER  
AN ILLUSTRATED ADDRESS BY DR PHILIP S HENCH

At the close of the Spanish American War thousands of American soldiers returned home to be received like conquering heroes. But in hospital some were of an enemy more powerful than any Spaniard. For disease, especially yellow fever, had killed more soldiers than had the bullets of the enemy. fever and wracked the dreaded "black

ernment, and with its Army of Occupation it sent physicians whose duty it was to control yellow fever, which had been endemic in Habana for about 300 years. Among these physicians were Major Gorgas, who was responsible for the health of the soldiers and civilians in the city of Habana, and Maj Jefferson R Kean, whose chief responsibility was the health of the American soldiers at Columbia Barracks on the outskirts of Habana. the

yellow fever continued to spread, and in May and June 1900, Major Kean

rate among the officers on the headquarters staff of Generals Wood and Lee was alarming. The clerks in General Wood's office burned on their desks sulfur candles as a prophylactic measure, but the candles burned in vain, and in the officers' "

"Here's to the ones who have gone

One of the earliest to go was M Kean. Because General Lee had already lost so many officers and men he ordered all those not immediately in charge of the sick to stay away from the sickrooms of those with yellow fever. Thus Major Kean could not visit Major Edmonds who lay sick unto death. But on the last bet off to the

the patient's room, he spent a last few minutes. During that short visit Major Kean was bitten by mosquitoes from the sickroom but thought little of it. But a few days later on June 21 Major Kean suddenly developed yellow fever.

As if to ridicule the puny efforts of the Army Medical Corps, the disease was now striking down the physicians themselves! Could nobody stop this evil thing! What was its cause anyhow! One of

within the incubation period of the disease. Thus today it is essential that the *Aedes aegypti* mosquito be kept under very close control.

Fortunately about the time jungle yellow fever was discovered, a very important observation was made in Brazil, namely, that it is possible to eradicate completely the *Aedes aegypti* mosquito in American cities and towns. It has been shown to be more economical over a long period of time to eradicate the mosquito than to maintain states of health.

which is larger than the United States, it is now free of this mosquito. It is a great pleasure to me to introduce to you from the audience this evening Dr. Waldemar de Sá Antunes, the Director of the National Yellow Fever Service of Brazil, who has been directing this work since 1941.

One of the problems the Brazilian health authorities have faced has, of course, been that of the reinfestation of the country along the

Americas. Dr. Heitor Prager Fróes, Director of the National Department of Health of Brazil, who presented this resolution in the name of the Brazilian Government, is with us this evening.

Following the approval of this resolution, a program has been drawn up and action started for the development of campaigns in South American countries, and it is confidently believed to be only a matter of time until *Aedes aegypti* will no longer exist in South America.

It should be mentioned at this point that the earlier experience in the eradication of *Aedes aegypti* was a very important factor leading to the eradication of *Anopheles gambiae* from Brazil.

It is now my very great pleasure to introduce to you Dr. Philip Showalter Hench. Dr. Hench has been associated with the Mayo Foundation since 1921 and has been head of its Department of Rheumatic Diseases since 1926. He was awarded the Heberden Medal and is honorary member of the Heberden Society, London, of the Royal Society of Medicine, London, and of the Liga Argentina contra el Reumatismo, Buenos Aires. Dr. Hench saw military service during World War II as colonel in the Army of the United States, and was Chief of Medical Service and Director of the Army's Rheumatism Center at the Army and Navy General Hospital. For many years his avocation has been the history of the Walter Reed experiments and the collection of related information and records.



the commonest ideas was that the mysterious cause of yellow fever arose like an evil spirit, an air borne poison from the tropical swamps. An Italian physician, Sanarelli, insisted it was due to a special germ which he had discovered. But nobody really knew the cause, and when a person died of yellow fever his home was often purified by fire to destroy his presumably infected furniture, clothing and other personal belongings, called "fomites." Thus hundreds of thousands of dollars worth of military and civilian equipment went up in smoke in an attempt to control the disease. But it was all in vain.

The old tragic story of yellow fever was being enacted again in the first year of the twentieth century as it had been enacted throughout the world for from 300 to 400 years of recorded history. Year after year yellow jack had invaded wide regions of the earth, spreading north and south, east and west, from its lair in the tropics. The West Indies were continually infected with the plague, and thence it

thick enough to bar it, and up and down the streets of villages, towns and cities of the South rode "the saffron horror," spreading fear and death.

From these doomed cities the panic stricken people fled by every available means. Some tried to escape by railroad, but often only the immune, who had previously survived yellow fever, were allowed to disentrain. More likely the refugees were turned back by fearful neighbors armed with rifles. When the trains stopped running, the refugees set out on foot. Fortunate were those who could flee in the

ways and when certain immune persons tried to enter a stricken city on errands of profit or even of mercy they also had to run the armed blockade.

For those unable to escape but unwilling to remain in their plague ridden homes only one recourse remained, mass migration into camps, generally set up on open high ground outside the city limits. Here mysteriously they usually found safety. To these camps were carried also the aged and infirm. As their tumbrils traversed the narrow streets acrid smoke rose from cans of tar set ablaze "to purify the death laden air." And like a grim salute to the dead that were and were to be, cannon boomed as helpless, ignorant, foolish men tried to stir up the stagnant air in a vain attempt to dissipate its mysterious poison.

During the great southern epidemics of the 1870's river steamboats shunned afflicted cities like Memphis. But to help the harassed population the steamboats paused above the city, then let loose barges laden

# WALTER REED COMMEMORATIVE MEETING

with food and supplies which floated downstream, were caught  
made safe at the otherwise abandoned wharves  
But before they could escape to safety hundreds of thousands  
veloped the disease, and thousands died Some had the comfort  
dying in their own beds surrounded by their grief stricken famil



But to many others even this comfort was denied stricken suddenly.  
they fell in the streets or in the parks, shunned by frightened passers  
by Soine, seeking mere shelter in lieu of a Samaritan crawled into  
abandoned cellars to die alone in the darkness, their bodies being  
discovered days later  
In this fearful manner great cities like Philadelphia and New  
Orleans were repeatedly attacked, to become desolate, shunned by the  
quick, abandoned to the dead and the dying Along the lengths of such  
great thoroughfares as Canal Street there fell a prolonged  
Overhead the smoke from the tar pots and cannons



appropriate shroud. Coffins multiplied and were quickly carried to cemeteries by hearse, by wagon, or by hand. Undertakers and grave diggers became totally inadequate, or fled for their own lives. Then the dead were abandoned or carried off by a surviving relative who may well have lost his whole family.

Such were the horrors of yellow fever prior to 1900, in which year long suffering Habana, now host to a conquering American Army, was stricken again. As Major Kean and other military personnel came down with yellow fever their recent victories "dried in their mouths." But on June 25, the fourth day of Major Kean's illness, Major Reed arrived in Habana, rushed to Major Kean's bedside, and in him saw his first case of yellow fever. Later that day Major Reed met with three others on the veranda of the officers' quarters at Columbia Barracks Post Hospital. The three others were Drs. James Carroll, Aristides Agramonte, and Jesse W. Lazear, and the four men thus ended their first day's work as the members of the United States Army Yellow Fever Board.

They first attempted to find Sanarelli's germ in the bodies of those sick or dead of yellow fever, but this search soon ended in failure. Perhaps after all no germ was responsible for the disease. Why in Quemados had the disease been so common? Why in the streets, striking first in the houses, then hopping around than crossing the affected street? Another curious fact was noted when Reed, Agramonte, and Lazear went to study an epidemic which broke out among the soldiers at Pinar del Rio. A soldier in a prison cell fell sick and died of yellow fever, but his cell mates, exposed to the same food and atmosphere, remained well. Could something have entered between the bars of the open window, struck one man down and gone away? Could yellow fever be caused by a winged agent? Could Dr. Carlos Finlay be right after all?

For 19 long years this kind, elderly Habana physician had been trying to convince his medical colleagues that yellow fever was caused by a common house mosquito. Absolutely sure of the truth of his doctrine, Dr. Finlay often sent reprints of his work first to his Cuban colleagues, later to high ranking American medical officers who replied with courteous little notes but did no more. Nobody believed

questionably induced or experimental rather than probably spontaneous. For Finlay's volunteers were not quarantined and those few who later developed yellow fever were believed (by everyone except

theory once and for all. They visited Dr. Finlay, who graciously gave all the help he could including a supply of mosquito eggs of the

1 Pinar del Rio  
sproved Finlay's

suspected species. Thereupon a momentous and heroic decision had to be made because no animal was then known to be susceptible to yellow fever. Human volunteers were required. Unwilling to ask others to do what they themselves would not do the Board decided to inoculate each other among the first. At this juncture Reed was unfortunately ordered to Washington to finish an important medical report. Carroll and Agramonte continued respectively their bacteriologic and pathologic studies and it fell to Lazear's lot to begin the mosquito work. This was fortunate because he of all the Board was most sympathetic to the Finlay theory. Indeed for some time Lazear had been trying (so far unsuccessfully) to prove a relationship between mosquitoes and yellow fever. Thus on the very day the Army Board was officially named in Washington, Lazear in Quezados, Cuba, was catching mosquitoes in the room of a patient with yellow fever and (as shown from notes in his laboratory notebook) was examining their bodies for agents responsible for the disease.

TO THE AMERICAN  
nothing happened

doubting, Carroll

\* In a few days

Carroll developed a severe and almost fatal attack of yellow fever. On the way from Carroll's bedside Lazear (without the knowledge of his colleagues) inoculated a scoffing volunteer soldier who "wasn't afraid of any little old gnat." When yellow fever hit him 6 days later this soldier became a very surprised hero whose widow later received his Congressional Medal. A memorial bridge in Grand Rapids, Mich., was named for him.

Having accomplished two very successful inoculations Lazear wrote his wife (September 8) "I rather think I am on the track of the real germ. But nothing must be said as yet, not even a hint. I have not mentioned it to a soul." How right he was was tragically proven by what happened 10 days later when he himself developed the dreaded disease. During his illness Dr. Lazear told two visitors, Drs. Carroll and Gorgas, that a few days before while he was feeding his mosquitoes on yellow fever patients at Las Animas Hospital, a stray

science. Such is the official version of this tragic incident. But I am about to tell you another version of the affair, one which was kept secret for 40 years, and which was not even known to Dr. Lazear's widow until I was permitted to tell her of it in 1940 through the courtesy of those who revealed it to me—Walter Reed's colleagues, Generals Truby and Kean, and Dr. Agramonte's daughter.

Reed hastened back to Habana (October 4) filled with mingled emotions. He was greatly depressed by Lazear's death, yet elated that success at last seemed at hand. But he was also confused. Why did the first nine inoculations fail and the next ones succeed? The second successful case seemed incontrovertible. Having been quarantined at the otherwise fever free post hospital the scoffing private (Private Dean—"case XY") had had no other conceivable source of infection than via the applied infected mosquito. But could one be sure that Carroll's disease had come from the experimental mosquito bite and not from some other source to which he might have exposed himself while going about town? And how could Lazear's tragic case be used to prove anything unless somebody knew what kind of a mosquito had bitten him?

## 1:

ing entries about Lazear's experiments. Reed eagerly studied these and of solution.

Reed

indeed cause yellow fever but only under certain special conditions.

By carefully noting the relative timings of each step in the successful and unsuccessful experiments it became obvious that patients with yellow fever have the agent or virus of their disease circulating in

infected mosquito' cannot transmit its deadly load or infect another person until the virus has had a chance to develop, or "ripen," within the mosquito's body for at least 12 days.

bitten patients too late) or had been bitten by "infected mosquitoes" which were still temporarily harmless because they had not been allowed to "ripen."

Thus Lazear's little notebook was vitally useful in solving one mystery but it posed another, for in it Reed found some incomplete entries which appeared to indicate that Lazear had secretly submitted himself to other experimental inoculations. Reed pondered long over these entries and then concluded that when Lazear was taken sick he must have worried lest his life insurance become forfeited if it became known that he had deliberately infected himself with a fatal disease. Actually this explanation was incorrect, Mrs. Lazear told me that Dr. Lazear left no life insurance.

But did he for some other reason at the last fateful hour withhold facts to protect his loved ones? Was this why he had told Gorgas and

decided to permit the official records to read that Lazear had become accidentally infected while in the performance of duty. Having made his quiet and heroic gesture Lazear had sought to carry his secret to a better world. Out of respect for the unspoken wishes of their friend, Lazear's colleagues have kept that secret all these years, Reed and others having carried it to their graves.

In so doing they eminently proved their loyalty to him. But it apparently disturbed them to deprive Lazear of a greater fame and in the following unpublished remarks of Agramonte I sense a wistful desire to rectify matters. At a Habana banquet in honor of Drs. Gorgas and Kean in June 1902 Agramonte's speech contained this tribute: "The one of us who from the very inception of our work so strenuously believed in the mosquito theory in connection with the propagation of yellow fever, the one of us who was best fitted by his training in the line of our investigation to successfully carry out the

Reed gave his "Preliminary Report" of this work at Indianapolis late in October but his report received little public credence.

Thus on November 2, 1900, an editorial in the Washington (D C) *Post* read: "Of all the silly and non-sensical rigamarole about yellow fever that has yet found its way into print—and there has been enough of it to load a fleet—the silliest beyond compare is to be found in the arguments and theories engendered by the mosquito hypothesis. The mystery remains, notwithstanding this 'board of Army medical men', whoever they may be. There is absolutely nothing in this mosquito hypothesis."

Knowing that a skeptical world would demand more proof than that afforded by these three successful but relatively uncontrolled inoculations, Reed now conceived and with Carroll and Agramonte executed a series of brilliant experiments which were to write the final chapter of this story. On the advice of Major Kean, Reed asked Gen. Leonard Wood, Governor General of Cuba, for money with which to set up an experimental camp and to pay such American and Spanish volunteers as might be secured. To the lasting credit of General Wood, who had himself been a physician, he promptly granted Reed's request and threw behind Reed all the authority of the Governor's high office.

Yellow fever was to be given away free with premiums of \$200. The victims could spend the money any way they wanted to—if they

survived; a rather big if, considering that the mortality rate of epidemic yellow fever was about 40 percent. But before any paid volunteers were secured two American soldiers, John J. Moran and John Kissinger, volunteered their services only on condition that they could do so without pay and in the interests of science. Legend has it that Major Reed, profoundly affected, rose and said, "Gentlemen, I salute you." Both Kissinger and Moran told me that actually the legend is not true, which Reed's widow and children were sorry to learn from me a few years ago. But as one writer said, "If Reed didn't salute them, he should have!" The world is still saluting them with many honors.

A specially guarded and quarantined experimental station named Camp Lazear was set up in a secluded spot a mile from Camp Columbia. The station was built for the purpose of studying the transmission of yellow fever.

Of this Reed wrote. "In my opinion this exhibition of moral courage has never been surpassed in the annals of the Army of the United States."

Then two small specially constructed wood buildings were erected. The first was called Building No. 1 or the "Infected Clothing and Bedding Building." It comprised one room, 14 by 20 feet, had only two small windows, and was heated by a stove to a tropical temperature. Three cots were set up and into this sweltering room were placed the offensive clothes and bedding of the volunteers who had been exposed to the bites of the mosquitoes.

Mr. Hanchett, who is one of your honored guests here, was one of the volunteers. He placed these offensive clothes around the walls and placed them on the beds, and then lay down to try to sleep on stinking pillows and sheets soiled with blood and vomitus. Stomachs rebelled, but spirits remained firm and not one of these volunteers developed yellow fever.

On the other side of the room, the "Infected Room," were placed the clothes and bedding of the volunteers who had not been exposed to the bites of the mosquitoes. These clothes and bedding were disinfected by a wire screen. On a cot in one side of this room, John Moran exposed his body to the bites of fifteen loaded mosquitoes let loose in the room. He was in the room only a little over an hour in all, but he promptly developed yellow fever, while other volunteers who stayed long hours on the other side of the screen where there were no mosquitoes remained well.

His yellow fever was a wonder to Carlos Finlay and to all the other scientists. On the next day, Sunday, the 10th of May, the day of the Lord's Eve, Reed in a mood of exultation and humble gratitude to God wrote his family a much quoted letter which has become famous.



*BUILDING NO. 1 Infected Clothing and bedding building  
Camp Lae Lae Dr. Nogueira Dr. Moran and Dr.  
Hench in front of building*



"11 50 p m, December 31st, 1900 Only 10 minutes of the old century remain Here I have been sitting reading that most wonderful work—La Roche on yellow fever, written in 1853 Forty-seven years later it has been permitted to me and my assistants to lift the impenetrable veil that has surrounded the causation of this most dreadful pest of humanity and to put it on a rational and scientific basis. I thank God that this has been accomplished during the latter days of the old century

"... The prayer that has been mine for 20 or more years, that I might be permitted in some way or sometime to do something to allevi

the 24 buglers, all in concert,  
beautiful it floats on the mid-  
night air."

Dr Finlay's 20 year old prayer had also been answered How

therein a stone, rough in appearance, I picked it up and with the assistance of my efficient and faithful co laborer Dr Claudio Delgado,

from the rough shell the stone to whose brilliancy none can now be blind"

But many were still blind, and most of the world still disbelieved On Saturday, December 22, 1900 (the same evening that those "in

which has continued to laugh at every solemn dogma proclaimed by the anointed . . .

"We shall waste no time on this new 'mosquito hypothesis' further than to suggest that it is as ridiculous as the broom, shovel, carbolic acid, and sewage hypothesis It occurs to us to say, only, that until some gentleman discovers the cause of yellow fever, other gentlemen will be wise to cut short their speculations as to its spread and propagation and devote themselves humbly to its treatment The latter is easy The rest of it is so far beyond the powers of the select"

After their brief pause for rejoicing Reed and his colleagues continued their work In the bodies of 12 more American and Spanish volunteers (Benigno, Fernandez, Precado, Martinez, Jernejan, Olson, Folk, Forbes, Andrus, West, Hanberry, and Sonntag) yellow fever



was produced at will, either through the medium of mosquito bites or by injections of infected blood or serum. Fortunately all these volunteers survived, thanks to the excellent care of Dr. Roger Post Ames. Their problem solved after just 8 months of work, the Board disbanded Camp Lazear on March 1, 1901. Now armed with precise knowledge, Gorgas within 3 months freed Habana of its age-old scourge. Later, with this and other knowledge, he made safe the Isthmus of Panama for the passage of the commerce of the world.

And what became of their battlefield, Camp Lazear? Reverting to commonplace uses it was lost for 40 years. Mr. John Moran, Mr. Luis Pogolotti (of Habana) and I hunted for it and rediscovered it in 1940. Building No. 2 is gone but Building No. 1 still stands, creaking with age and sleeping in the Cuban sun. At its back is an encroaching quarry, in front a field of corn.

I revisited it a few weeks ago (March 1948) with Mr. Moran and Dr. Pedro Nogueira. You will be interested to know that the Cuban Gov-

men who banded  
for one country  
tries. These 25  
men included 3 Cubans, 16 Americans, 1 Englishman, 1 Irishman and 4 Spaniards. Some were Catholic, some were Protestant, some were Hebrews. United in a common cause they demonstrated magnificently the human capacity for greatness and courage. It is such as they who reassure us of the inherent decency and dignity of man.

## COMMEMORATION OF THE FIFTIETH ANNIVERSARY OF THE DISCOVERY BY RONALD ROSS OF THE METHOD OF TRANSMISSION OF MALARIA

A meeting was held in the departmental auditorium at 8:30 p. m. Friday, May 14, 1948, to celebrate the fiftieth anniversary of the discovery by Ronald Ross of the method of transmission of malaria.

uments and instruments relating

his microscope. In a reserved section at the front of the audience were seated some 30 distinguished scientists and sanitarians who had done important work in the investigation and control of malaria.

After music by the United States Army Band, the meeting was opened with introductory remarks of the chairman, Prof. George Macdonald, director of the Ross Institute of Tropical Hygiene, England. His opening speech was followed by incidental music by the

**Army Band** The chairman then introduced Dr Paul F Russell, International Health Division of the Rockefeller Foundation, who, in turn, presented the orator of the meeting, Sir Malcolm Watson, emeritus director of the Ross Institute

After the oration of Sir Malcolm Watson on "Sir Ronald Ross," the United States Army Band played the British national anthem, in recognition of the nationality of Sir Ronald Ross, and then the national anthem of the United States This concluded the exercises.

The addresses of the several speakers will follow

**OPENING SPEECH BY THE CHAIRMAN, PROF GEORGE MACDONALD,  
DIRECTOR OF THE ROSS INSTITUTE OF TROPICAL HYGIENE,  
LONDON**

Sir Malcolm Watson, Honor Guests, Fellow Delegates, and Mem

manship of Sir Eric McFadyen some long time ago to organise a suitable celebration of the jubilee of his discovery We originally intended that that celebration should be held in London We received the offer of its celebration at this conference, and I wish to make clear our committee's gratitude to the Government of the United States and the organisers of this conference for having made such an ap

re made

Their

names are listed on the program you have For me to attempt an evaluation of their individual contributions would surely involve me in faults of appraisal which would be unjust I shall let their own works, which are well known to all of you, speak for them I must, however, refer individually to one, to say what a great pleasure it is to have with us that original pioneer in the application of Ross's discovery, and now the honored veteran of tropical hygiene, Joseph Augustin LePrince

Our guests include men who have devoted themselves to the study of the parasitology of malaria, studies which have recently culminated in the discovery of the pre-erythrocytic stages of mammalian *Plasmodia* but which had, before that, already opened up a great field of knowledge . . . malaria Others by the study . . . outcome of the Far Eastern . . . further advanced our knowledge of the prophylaxis and treatment of malaria Entomologists have, by their study of the taxonomy, bionomics, and physiology

was produced at will, either through the medium of mosquito bites or by injections of infected blood or serum. Fortunately all these volunteers survived, thanks to the excellent care of Dr. Roger Post Ames. Their problem solved after just 8 months of work, the Board disbanded Camp Lazear on March 1, 1901. Now armed with precise knowledge, Gorgas within 3 months freed Habana of its age old scourge. Later with this and other knowledge he made safe the I.

commonplace uses it was lost for 40 years. Mr. John Moran, Mr. Luis Pogolotti (of Habana) and I hunted for it and rediscovered it in 1940. Building No. 2 is gone, but Building No. 1 still stands, creaking with age and sleeping in the Cuban sun. At its back is an encroaching quarry, in front a field of corn.

I revisited it a few weeks ago (March 1948) with Mr. Moran and Dr. Pedro Nogueira. You will be interested to know that the Cuban Government has now designated this "old warrior" as a national monument and we are hoping that it will be properly preserved.

In these days when man's inhumanity to man is still so pathetically

men included 3 Cubans, 16 Americans, 1 Englishman, 1 Irishman and 4 Spaniards. Some were Catholic, some were Protestant, some were Hebrews. United in a common cause they demonstrated magnificently the human capacity for greatness and courage. It is such as they who reassure us of the inherent decency and dignity of man.

## COMMEMORATION OF THE FIFTIETH ANNIVERSARY OF THE DISCOVERY BY RONALD ROSS OF THE METHOD OF TRANSMISSION OF MALARIA

A meeting was held in the departmental auditorium at 8:30 p. m., Friday, May 14, 1948, to celebrate the fiftieth anniversary of the discovery by Ronald Ross of the method of transmission of malaria.

A special committee was organized by a scientific expert, with historic documents and instruments related to Ross and his work, including his microscope. In a reserved section at the front of the audience were seated some 30 distinguished scientists and sanitarians who had done important work in the investigation and control of malaria.

After music by the United States Army Band, the meeting was opened with introductory remarks of the chairman, Prof. George Macdonald, director of the Ross Institute of Tropical Hygiene, England. His opening speech was followed by incidental music by the

**Army Band** The chairman then introduced Dr. Paul F. Russell, International Health Division of the Rockefeller Foundation, who, in turn, presented the orator of the meeting, Sir Malcolm Watson, emeritus director of the Ross Institute

After the oration of Sir Malcolm Watson on "Sir Ronald Ross," the United States Army Band played the British national anthem, in which the British flag was hoisted, and then the national exercises.

OPENING SPEECH BY THE CHAIRMAN, PROF. GEORGE MACDONALD,  
DIRECTOR OF THE ROSS INSTITUTE OF TROPICAL HYGIENE,  
LONDON .

Sir Malcolm Watson Honor Guest Fellow Dealmaster and More

whole economy and culture of the world for the good in a manner as decisive as that of the invention of printing centuries before

The Ross Institute in London, which is directly charged with the perpetuation of Ross's memory, formed a committee under the chairmanship of Sir Eric McFadyen some long time ago to organize a suitable celebration of the jubilee of his discovery. We originally intended that that celebration should be held in London. We received the offer of its celebration at this conference, and I wish to make clear our committee's gratitude to the Government of the United States and the organizers of the conference.

re made

Their

Names are listed on the program you have. For me to attempt an evaluation of their individual contributions would surely involve me in faults of appraisal which would be unjust. I shall let their own works, which are well known to all of you, speak for them. I must, however, refer individually to one, to say what a great pleasure it is to have with us that original pioneer in the application of Ross's discovery, and now the honored veteran of tropical hygiene, Joseph Augustin LePrince.

Our guests incl

the study of chemotherapy made the successful outcome of the Far Eastern War possible, and have since then further advanced our knowledge of the prophylaxis and treatment of malaria. Entomologists have, by their study of the taxonomy, bionomics, and physiology

of mosquitoes, made an effective strategy against them possible, and others have concerned themselves in the production of new insecticides, the advent of which is as important to the malarialogist as

ago gained for the good of man, and that the pioneer in its application deserved credit equal to that granted to the pure scientist. I am, therefore, particularly glad to welcome among our guests some of the pioneers of malaria control in recent years, men who, by the expulsion of *Anopheles gambiae* from Brazil and by the subsequent schemes of eradication of mosquitoes from other lands, have set examples which will shape the pattern of our behavior in future years.

Our orator, Sir Malcolm Watson, is most welcome. Since 1900 he has been the disciple, colleague of Ross, of whom he will speak expressing our sense of honor  
duction to Dr Paul F Russell

(Interval for music)

I introduce Dr Paul Russell to you with great pleasure, but some hesitation. With great pleasure because he is an old and much respected friend, with some hesitation because there can be few people in this auditorium to whom it is necessary to introduce him. Through his work, his wide travels, and the charm of his company, he has gathered an almost unique circle of friends throughout the world. That circle included Sir Ronald Ross during his lifetime and has for many years included Sir Malcolm Watson.

Dr Russell's participation in this celebration is particularly happy on account of his great knowledge of malaria and its control, and because he is a true follower of Ross, who epitomized his whole purpose in life in the words, "I did not do this work on malaria in the interests of zoology, but in the interests of practical sanitation."

Russell has the same purpose, the cultivation of the field of knowledge for the good of man. The exact form which it was to take

with Samuel Taylor  
aids Settlement Rural  
the study of malaria

and its impact on man, in order to develop methods for its control which were within the economic reach of poverty stricken rural populations. It is right here to recall that at that time, over 20 years ago, it was commonly the belief that the mosquito was the only cause of malaria.

be a p  
Rus  
in 192

ganization—the rural health centre, now generally recognized as ideal—on which any programme of disease prevention must be based. In the Philippine Islands, in 1923-34, he carried out researches into

the epidemiology of malaria, in the course of which he brought out for the first time important characteristics of the carrier, and thereby narrowed the field of attack necessary for its control. By investigations into methods of control, particularly the use of Paris green, he showed how that attack could be made. And through his own actions and the pupils he taught, he initiated rural malaria control in those islands.

In India in 1934-42, he carried out a long series of researches which ended in the demonstration that by destruction of adult mosquitoes the peasantry could be protected from malaria at a cost as low as 7 United States cents per person per year. That work, which marked the attainment of his object and seemed to many of us the achievement of the impossible, was brought to an end by war. Its exact method has since been outmoded by the production of new insecticides, but it set the pattern which is now at last being applied to the Indian countryside to the incalculable benefit of that country.

In war, his talents were used in the service of his country and its allies. In the South Pacific he laid down the principles which led to the conquest of the malaria which might have made victory impossible. In the Mediterranean area, he will be especially remembered for his work in restoring the destroyed malaria control system in Italy. By that work, he protected the allied forces, averted a major tragedy to the Italian people and restarted a control system, the subsequent achievements of which have been described to us at this Conference. In America, he distinguished himself as a teacher and as the author of a valuable book on malariology.

He now enjoys the position of authority to which his experience entitles him in the counsels of the United States, the Rockefeller Foundation, and the World Health Organisation. It is with pride, as well as pleasure, that we call Paul Russell a friend.

#### INTRODUCTION OF SIR MALCOLM WATSON BY DR PAUL F RUSSELL, INTERNATIONAL HEALTH DIVISION OF THE ROCKEFELLER FOUNDATION

Mr. Chairman,  
I  
It kings that Elisha said unto Elijah, "I pray thee let a double portion of thy spirit be upon me." Soon thereafter, "Elijah went up by a whirlwind into heaven. And Elisha saw it, and took the mantle of Elijah that fell from him and smote the waters." (2) 1st anni

Watson

Infirmary He was on his way to becoming an eye specialist, but a trip around the world as a ship's surgeon and an aversion to cold weather turned his thoughts toward the tropics. So in 1900, with a Diploma in Public Health from Cambridge, and armed with courage and enthusiasm, with a keen mind and a sound body, developed by riding and yachting, Watson left Scotland and moved 7,500 miles to Malaya. There he served 8 years as an officer in the Government Medical Service and there for 20 more years he carried on an active private and consultant practice in curative and preventive tropical medicine, especially in all phases of malariology.

Malaya at the turn of the century was a land of promise severely blighted by malaria. Perennial humid heat in this well watered land provided favorable conditions for *Anopheles* mosquitoes, which at that time — — — — — crocodile, king Malay States, with — — — — — of the

population died of malaria in a single year. Two months after its opening, the important Port Swettenham nearby was ordered to be closed because of malaria. Klang merchants suspended business for days to perform ceremonial rites that the malaria dragon might be appeased and dissuaded from taking ever more human sacrifice (3).

Such was the menacing situation which faced the young district. Many were

on local rubber estates as many as 150 in every 1,000 laborers were dying of malaria in a single year was a challenge which Watson accepted and magnificently met. He kept Port Swettenham open, he lifted Klang's malaria burdens, and he contributed mightily to the development of the country. Without malaria control there could have been no Malayan rubber industry.

Why did Watson succeed so notably? Well, first, in contrast to most young physicians of that period, Watson believed his responsibility to involve "doing more than remaining in hospital all day treating patients, since to this there could be no end, if steps were not taken to prevent infection of the population" (3). This strong belief in preventive medicine was the foundation of Watson's work.

Secondly, Watson had read that malaria is transmitted by *Anopheles* mosquitoes and he knew that Ross was preaching mosquito reduction as a control measure. He also was aware that few scientists agreed with Ross. Indeed, the latter wrote in 1901 that he doubted if except in Hong Kong and Lagos a single life anywhere had been saved by attention to his mosquito malaria theory (4). Watson knew that the

— — — — — had some success in commended ignoring mosquitoes. kably well informed, far more so than the average Medical Officer of his time.

In the third place, while Watson has respected the written he never has worshipped it. He always has been one to thrust himself He went out of library and ward into the countryside to observe their habits, and to study the topography of the land. Research was combined with control. Watson became a practical malarialogist.

Although never before to his knowledge had he seen living anopheline larvae, Watson soon found them. In fact, they were everywhere in seepage and swamp, in hill stream and coastal river, in sunny rice field and shady jungle, multiplying prolifically the year round, without climatic check of a cold season or of one too hot or too dry. The Malayan species were surprisingly versatile and their natural history presented many paradoxes. For example, flooded ricefields teeming with *Anopheles* larvae were not malarigenous, yet sly seepages with relatively few mosquitoes were deadly. Letting sunlight into a swamp would drive away one malaria vector but attract another. All in all, the problem was huge, but Watson, having obtained first hand information, made a profound decision in that early day. He decided to try Ross's mosquito reduction method of malaria control and he proposed to do this principally by works of a substantial nature, such as drainage and fills to eliminate mosquito breeding places.

For the next quarter century Watson fought *Anopheles* mosquitoes in Malaya with consummate originality and skill, and greatly stimulated others by his brilliant example. The total result of cooperative efforts was such that Sir Ronald Ross after a visit in 1926 described Malayan malaria control as "the greatest sanitary achievement ever accomplished in the British Empire" (5).

Watson determined many points which have had basic importance. For instance, he was first to demonstrate that one does not need to destroy all anophelines to control malaria in a community. Find out which species carries malaria locally and concentrate on it, using methods which take full advantage of the natural behavior of the vector. By this principle of species sanitation Watson brought a new range of many areas where previously the situation had been considered hopeless. Malarialogists the world over have confirmed the soundness of this fundamental concept which originated with Watson.

Watson was first to take malaria mosquito control into rural areas, extension, the feasibility of which even Ross had doubted (6). He was also first to point out the possibilities of biological or chemical mosquito control, first to use subsoil drainage of ravines for malaria control, and first to use larvicides in running water. Nor will it be forgotten that Watson has greatly encouraged and helped by visiting the Netherlands East Indies, the Balkans, India, the Americas, and other areas. From 1928 to 1942 at the Ross Institute in London as Principal of the Malaria Department and then



as Director of the Institute, he taught hundreds of lay and medical students the principles of malaria control. Moreover, his account of "The Prevention of Malaria in the Federated Malay States" (3), published in book form, had wide and profound influence in the development of mosquito abatement. Another textbook entitled "Rural Sanitation in the Tropics" (7) and many scientific papers also have had lasting value.

In 1914 the Rubber Growers' Association presented Watson with an honorary gold medal. In 1924 he was knighted by the King of England and in the same year was made an honorary Doctor of Laws by the University of Glasgow. In 1927, Sir Malcolm was awarded the much treasured Stewart Prize of the British Medical Association and in 1928 the Sir William Jones Gold Medal of the Asiatic Society of Bengal. The Mary Kingsley Medal of the Liverpool School of Tropical Medicine was conferred on Sir Malcolm in 1934 and the notable Albert Medal of the Royal Society of Arts in 1939. Sir

laurels. For example, during the past three years he has spent many hours crawling through mines getting first hand information which would enable him to devise practical dust control methods for the prevention of silicosis. In other words, Sir Malcolm still retains the vigorous mental enthusiasm of his younger days and he still believes in direct action.

Mr Chairman, Members of the Congresses, Distinguished Guests,

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RONALD ROSS COMMEMORATIVE MEETING  
SIR RONALD ROSS: AN ORATION BY SIR MALCOLM WATSON

*"Ring out old shapes of foul disease,  
Ring out the narrowing lust of gold,  
Ring out the thousand wars of old,  
Ring in the thousand years of peace"*

As the bells of Christendom rang out the nineteenth century, they proclaimed two discoveries which were to save millions from old shapes of foul disease", for in India between 1897 and 1898 Ronald Ross discovered that malaria was spread by the *Anopheles* mosquito and in 1900, in Cuba, Walter Reed and his colleagues showed how yellow fever was spread by the mosquito known as *Aedes aegypti*.

You have honored me with an invitation to speak to you this evening in commemoration of the jubilee of Ross's discovery. He always insisted that his work was done not for the sake of science but for humanity, and I am sure nothing would have delighted him more than to hear that we have used his discovery with profit for that purpose. He was indeed a "Helper of the World and a Friend of Man", and tonight I feel we stand at the bar of history to give an account of our stewardship.

So my main purpose is to tell you where and how we have used the discovery to triumph over malaria, and particularly of the six outstanding achievements, to tell where for long we were slow in starting, and why, so that we may be reminded of the danger that besets the discoverer and the new idea however beneficial it may be to mankind.

Before doing this I would briefly remind you of Ross's eight years of research before success came—and in the course of what I say you will form some picture of one whom Carlyle would have called a 'Great Man', and 'The Hero as Scientist', of his youth before the discovery, of his career as a man after it. For nearly 30 years it was my privilege to call him friend.

### THE YOUTH ROSS

As a medical student Ross was a failure. His interests were wider than the medical course, for as a youth "he determined to acquire mastery of all the arts of man and he did in fact acquire a mastery of mathematics and music, became a composer of songs, a poet of great eminence, a painter of distinction, and a scientific researcher of the very first rank." That is how his friend Mr John Ruskin, the Poet Laureate, described him in 1936.

Ross, describing these things in a discourse to the Royal Institution in 1920, declared

Do you really imagine that science is concerned only with the discovery of petty utilities, Art with the discovery of new tricks of technology, and literature with mean books written by, for, and about mean

people? I say not art for art's sake, or science for the sake of science, but both for humanity"

Here he stated the guiding principle of his life, and he pursued his principle at all cost. Mr. Masefield said of Ross on the same occasion, "Ronald Ross was a man who was not a man for the sake of a man."

book, *Ronald Ross, Discoverer and Creator*, which will interest many, for Mr. Megroz wrote from personal knowledge of Ross. They will be found in the exhibition.

### THE MALARIA DISCOVERY

For four years Ross worked on wrong lines and "fell into error," as he tells us. Then in 1895 he met Patrick Manson, a Scot despite his Christian name. Manson, the "Nestor" of the younger workers in tropical disease, explained to Ross his version of the idea that mosquitoes spread malaria. He believed, as a result of work he had done in China in 1878, that the mosquito became infected with malaria when it bit a man with that disease, that subsequently it died on water, and that man became infected when he drank the water. Manson advised Ross "to follow the flagellum" when he returned to India, for he correctly believed that this "flagellum" was the form of the malaria parasite destined to infect the mosquito. Unfortunately the "flagellum" behaved like the giraffe when it said to the leopard as he moved into the forest, "Now watch. One two three—and where's your breakfast?"

It was two long years before Ross discovered what had happened to the "flagellum," for it was even better camouflaged than the giraffe. He was looking for a fine colorless thread quivering its way among blood cells. What he found on the 20th of August 1897 among the fibers of the wall of the mosquito's stomach was a tiny cell with little black spots like little beady eyes staring up at him with not the quiver of an eyelid. He recognized it as the malaria parasite. He called the 20th of August, "Mosquito Day." That night he added to his poem *In Exile* the following well known lines

*"This day relenting God  
Hath placed within my hand  
A wondrous thing, and God  
Be praised At His command,  
Seeking his secret deeds  
With tears and toiling breath,  
I find thy cunning seeds,  
O million murdering Death!"*

His work was now interrupted for the second time. There was so much delay before Manson persuaded the India Government to put Ross on special research on malaria in Calcutta. With the key to the problem in his hand, he finished the research in a few months, and on the 9th of July 1898, showed that the malaria parasite, after strange developments in the mosquito, returned to man as it had come from him, through the bite of the insect.

### TRIUMPH

The measure of Ronald Ross's triumph is in the record of what men have done since with the power he put in their hands. In the six outstanding achievements of which I shall speak the work of Walter Reed on yellow fever and the work of Ronald Ross on malaria came to fulfillment.

### HABANA AND PANAMA

After the two discoveries that malaria and yellow fever were carried by mosquitoes the United States Government was "quick off the mark" first in Habana in 1900, then in Panama in 1904 and in continental United States of America in 1912.

In 1913 I spent about 3 weeks on the Canal Zone, and walked over the whole area under sanitary control. Major (now General) Noble was kind enough to accompany me on many occasions or arrange for an inspector to go with me. Whatever I wanted to see I was shown, and there was the frankest discussion and criticism of their own work by the department of sanitation. Each night I made careful notes of what I saw and embodied them in a book—*Rural Sanitation in the Tropics*.

At Panama between 1881 and 1889 the French had died of yellow fever and malaria as if mown down by machine guns. In 1913 there had been no case of yellow fever for 7 years and malaria was represented by about one half of 1 percent of the labor force per week. The labor force was as healthy as if they had been living in a temperate region, and the greatest engineering work the world had seen was moving smoothly to its near completion. So I feel there is some justification for the remarks I am about to make and the conclusions I drew. First That William Crawford Gorgas was the greatest sanitarian the world had seen.

Second His was pioneering work.

Third Becoming a commanding general, he had been on active service in the deadliest of campaigns first in Habana in 1898, then in Panama from 1904 to 1915, and in the United States, South America, Europe until his death in London on the 4th of July, 1920. I know of no medical man who has borne so heavy a burden for so long a time with such uniform and complete success, not always with the credit he was entitled to expect.

Fourth He never lost a battle.

Presenting Gorgas for an honorary degree at Oxford in England, the orator said:

"The reputation of Gorgas as a scientist has been challenged in certain quarters, in view of the fact that he was not responsible for the actual discoveries without which his work could not have been done. For this he needs no defense. Science and art are at their greatest when they join hands, and the man who acts as a link between

its application. But even when research has been undertaken with the sole aim of finding the cause of an epidemic fever or the source of an infection, the successful investigator would often cut a poor figure as the organizer of an expedition to stamp out the scourge in the light of his discoveries. It is not only as a scientist but as a leader of men, as the hero of at least two of the most successful campaigns ever waged, that the name of Gorgas will always be gratefully remembered."

Honour to whom honour is due

In Habana Gorgas found in Dr. Henry Rose Carter, an officer of the United States Public Health Service, not merely a scientific ad

of malaria in the United States

An army requires more than a general staff, there must be efficient field officers. First in the Mission at Habana, and later in Panama Gorgas gave Le Prince the task of field organization and supervision in developing the attack against the mosquitoes of yellow fever and malaria. Subsequently at Carter's insistence he became the chief field

son of gradu in 189 fever in its history since 1762

Panama  
"a title  
ince, the  
shire mother,  
y, New York,  
learned yellow  
the first time

in its history since 1762

His chart of the number of mosquitoes swept up from the floors

Le Prince suggested he might wipe out malaria while they waited

ed malaria  
technique  
is totally

different

It was all pioneering work, but Le Prince had the essential qualities—imagination, invention, energy, organising power. Like Admiral Nelson, he had a "blind eye" when a job had to be done in a hurry without approval from above. He had also an insight into the minds of mosquito and man. Le Prince knew that yellow fever did not automatically disappear when a town in the American tropics

or he knew what  
looked better and  
when washed in

pipe water, and they were ready to fight for woman's right to look her best!

At the Seventh Congress of the Far Eastern Association of Tropical Medicine held at Calcutta in 1927 resolutions were passed on the control of malaria. Among these was the following

"The Congress desires to stress the need not only of thoroughly trained malarial research officers, but of expert malarial engineers in whichever type of malaria prevention is at stake."

I have heard with pleasure that a university has conferred the degree of doctor of science on Mr. Le Prince, D.Sc., the national and exemplary pleasure to record

### CRISIS IN THE WEST

"I am sorry for you tonight, Mr President," wrote his friend Dr Alexander Lambert to President Theodore Roosevelt in 1905. "You are facing one of the greatest decisions in your career. Upon what you decide depends whether or not you are going to get your canal. If you fall back on the old plan, the canal will fall to the ground."

There was only one way of controlling yellow fever and malaria, and that is the eradication of mosquitoes. But it is your canal, you must do the choosing, and you must choose tonight whether you are going to build that canal."

It was a critical moment for the canal. The Canal Commission had recommended that Gorgias should be dismissed and "replaced by a man with more practical ideas."

To add to the President's difficulty, Gorgas's dismissal was supported by the then Secretary for War

With my own ears I heard Gorgas tell that to a great congress of physicians and surgeons here in Washington in 1913

Indeed it was a critical moment for more than the canal. The wrong decision would have set back the control of yellow fever throughout the Western Hemisphere, and might have led to its spread to Asia. When yellow fever struck Memphis, Tenn., in 1878, it killed 4,200 out of 6,000 whites between the 16th of August and the 27th of October. The life of the city was paralysed, and all fled who could. In Asia there are more than 800 million nonimmune people, and there is no reason to think that their mortality would be less than that of Memphis in 1878 were a yellow fever epidemic once started. Well might Sir Patrick Manson describe an outbreak of yellow fever in Asia as a world disaster of appalling magnitude. There is still a danger to Asia from yellow fever spreading from Africa, as I pointed out officially to the Government of Malaya in 1914 and said in my *Rural Sanitation in the Tropics*, published in 1915.

The President made the right decision. Gorgas remained, and was promoted to membership on the Canal Commission.

#### ROCKEFELLER FOUNDATION

The world has reason to recall with gratitude the names of many great citizens of the United States of America.

In creating the Rockefeller Foundation and the International Health Board, Mr. John D. Rockefeller planted a tree of life and 'the leaves of the tree were for the healing of the nations'. It would take volumes to record all its deeds of mercy. Here I can speak only of its work against malaria. In addition to that done in the southern States of the United States of America, it taught Europe and the Malaria Commission of the League of Nations that it was cheaper to prevent malaria than to cure it, and the cooperation of Hackett and Missiroli cleared malaria out of the Roman Campagna where it had held sway for 2,000 years.

Its high water mark was in eradicating *Anopheles gambiae* from Brazil and from Egypt. There is nothing more brilliant in the history of the prevention of malaria than that described by Soper and Wilson in the book *Anopheles gambiae* in Brazil, 1933-1940. They threw this African invader out of Brazil and saved the whole Western Hemisphere. It prevented the deaths of millions of people which the spread of this winged terror would have made inevitable. Indeed, it stands out as one of the greatest sanitary achievements of all time.

And now we must turn to India, the birthplace both of Ross and of his discovery.

## CRISIS IN THE EAST

pende whether or not millions of your fellow men will live or die  
The decision must be yours. You must make it today."

terview lasted 3 minutes

Ross left India on the 24th of February 1898, weary and worn from his long researches. But the cool weather and rest on the voyage revived him and he thought as he sailed along the sunny Spanish shores

"In two years we shall stamp malaria out of every city and large town in the tropics—at least if they possess sanitary departments as in British possessions. And this is not the dream of a visionary. My experience of sanitation in Bangalore has taught me what few medical men possess, a thorough knowledge of town management, and I knew what I was talking about—sanitary organisation, town cleansing, sanitary engineering, houses, yards, sewers, official procedure, and the rest of it."

He had the further qualification of having studied and taken the diploma of public health of London, and having studied the new science of bacteriology under Klein on his leave in 1880.

The voyage had revived his hope, and hope like

" . . . love resembleth

How descriptive of Ross's life Shakespeare's words are—with its hope and despair, its joy and sadness, its tragedy, triumph, and defeat. But, thank God, it was triumph before the end.

On his arrival in England in March 1898, he took a poorly paid appointment at Liverpool instead of starting practice in London, so that he might continue his research in malaria and make a start on prevention. In the same year, he confirmed his discovery in the African *Anopheles* at Sierra Leone, and on the 2d of July 1901, started with money provided by the government would continue. But it did not do so, and



of this experiment was near Lahore, India. The experts in India concluded that antilarval operations were "difficult," "ineffectual," "useless," and "futile."

The one man essential for success—Ronald Ross with his practical experience—was 6,000 miles away; nor was there an engineer like Le Prince of Panama or Harold Gray of California in the team, or the result would have been very different.

In a letter to me in 1904, Ross wrote "I fear that experiment will put back the hands of the clock in India for another generation." It was to do so, and not merely in India but in extensive regions of the

For

made in the Calcutta office

In Macedonia, in 1917-18

British Army "Malaria

tion," is printed in the Official History of the War

In Ceylon, in 1934-35, a malaria epidemic killed 80,000 people. For 800 years malaria had made uninhabitable about one third of the island, in which the remains of a great civilization lay buried under a mantle of green jungle.

When the last war broke out, the West African ports were as malarious as ever. So when the allied forces landed to make air bases for the Middle East, some 80 percent of the men became infected. Brazil pointed out that these ports were a danger to the whole Western Hemisphere in exporting *Anopheles gambiae* and other pests, and she was entitled to do so, for had she not already thrown *A. gambiae* out of her own territory?

### THE MAN ROSS

By 1904 his critics were in full cry. Their arguments were

1. It was impossible to reduce mosquitoes

2. Mosquitoes like Nature abhorred a vacuum—they would flow into any area in which they had been destroyed—if you had managed to do the impossible.

—not the *Anopheles*

better

ending

fter

And all this in spite of the well known facts—indeed, proved by Dempster's brilliant work in India 100 years ago—that malaria was a very local disease, also that well fed and well housed British and American soldiers in malarial regions suffered severely from malaria, and that successful mosquito malaria control had been done at Habana, Panama, Ismailia, Malaya, and elsewhere.

hid these qualities under a mask of indifference, it was said that he was not made for commerce with his fellows, and even that he was not a scientist—this latter by a well known scientist to myself

mistakes, Ross forgave him

On the other hand, he could not have been foolish any more ago

A trickster he never forgave, unhappily 'the cunning keep the crown', for in England as in Denmark, 'A man may smile and smile and be a villain'

Unhappily, too, his critics were in the inner lines "After his discovery, the rest of his life was devoted to enlarging and completing what he had begun. It was passed in an obscurity which is likely to occasion surprise in the future as well as regret," wrote

But the day of tribulation for the tribes of the Philistines was nigh.

*"Fear not Unsheathe the naked falchion. Try  
The end For in the end, who dares deny  
The utter truth will slay the utter lie"*

—R. R. 1890-93.

THE ROSS INSTITUTE OF TROPICAL MEDICINE AND HOSPITAL FOR  
TROPICAL DISEASES, PUTNEY, LONDON

A proposal to establish a Ross Institute, made in the *Times*, London on the 23d of June 1923, was backed by many of the most dis

tinguished men in every sphere of life. At the end of 1925 the Institute was opened, and under the wise guidance of Sir Charles McLeod, Sir Austin Chamberlain, Mr A. Chester Beatty, and Sir

Ross' death it has had two directors, myself until 1943, and Dr G Macdonald since. An important contribution to its success has been the Industrial Advisory Committee, which meets in the city of London for the convenience of its members. Its proceedings, which are widely circulated to the press as well as to its members, set out that "The Ross Institute Industrial Advisory Committee was formed in 1928 to keep Industry in touch with Science to make the Tropics Healthy, and to Expand the Markets of the World."

It has been fortunate in its chairmen, Mr A. W. Still, a past president of the Institute of Journalists, Mr G. H. Masefield, a brother of the poet laureate, and Mr A. Wigglesworth, a leader in the African sisal industry. Of great value, too, has been its Malaria Course for Laymen. Nearly 1,000 men from many parts of the tropics, and of every occupation, took this course between 1928 and 1938.

Such was the success of the Ross Institute in its work overseas that it received and accepted in 1933 a proposal for amalgamation with the London School of Hygiene and Tropical Medicine—itself founded

The Ross In-  
stitute's work in the  
at the school  
Foundation,

nonpolitical and nonpartisan, have been welcomed by kings and princes, by governments, by great tropical industries, and by societies of peasants and humble folk. Today they are working in most tropi-

## MALAYA

In 1926, Ross visited Malaya. I had the pleasure of driving him for hundreds of miles and showing him work for the prevention of malaria. He was acclaimed and feted everywhere, for the people of Malaya, official and unofficial, had seen the benefit of malarial pre-

By 1901

greatest sanitary achievement ever accomplished in the British Empire.

In 1875 the British entered Malaya at the invitation of the Sultan to stop civil war and piracy.

Not only was Malaya advanced in sanitation, but many tributes

here they had a great many races who were living happy and contented lives in spite of  
 ture They were together  
 genuinely friendly feeling

soil was fertile for their happy life, which was necessary for the cultivation of the friendly feeling " He also referred to the value of science

From Malaya, Ross traveled to Calcutta There a Memorial Gate at the Presidency Hospital, where he had completed his discovery in 1898, was opened by H E Lord Lytton, Governor of Bengal, after an interesting address by Sir John Megaw—later the distinguished director of Ross' old service

### AFRICA

Ross was greatly interested when, in 1929, the Ross Institute began work on Mr A Chester Beatty's group of copper mines in Northern Rhodesia, for he had not forgotten the neglect of his work in West Africa Mr C R Harrison, originally a rubber planter in Malaya, organized the antimalarial work, mainly by drainage and oiling, producing an immediate effect on the sick rate and death rate

When I visited Rhodesia in 1930, a senior government medical officer said that as a mosquito could fly 5 miles and one mosquito could

i met him again he said so

In this part of Africa—600 miles from the Equator—sanitation on the mines included the control of the two great African carriers of malaria, *Anopheles* and *Culex*, living in the excrement and their holes from mud huts which they shared with a whole host of animals and parasites—fleas, lice, ticks, rats, mice, and snakes—brought with them

healthy as if they lived in a temperate region. Very remarkable, was how the African women rose to their new surroundings—a garden city, as I described it in a special article in the *Times*, London, on February 10, 1940. "But already the copper mines have shown the African what a better standard of life means, have stimulated the woman to seek it for herself and her family, and, not least important, have taught her to live it."

At a recent meeting of the Royal African Society in London there was a rather inconclusive discussion on the problem of incentives and how to induce the African to learn European languages and write other, how to get

enough to pay for social services, instead of having for his first objective officer said three lines the nomic, and they

had been taken in that order. With all due respect I would suggest that this is the wrong order, that a lesson be taken from Malaya, that the copper mines should teach to all in Africa less of an inferiority complex to pests and parasites, and that the whole African social structure should be built on a "healthy village" such as I suggested

tallurgy, London, have not seen any surplus energy,

who were sodden with disease. And it seems to me that to expect it from the African can only come from never having seen such a change in a man's physique and energy as occurs on the copper mines after a year's residence or on an estate in Malaya. I commend these matters to those responsible for the African Continent.

### INDIA

In 1930, a branch of the Ross Institute was founded in India through funds provided by Sir Charles McLeod and his friends—Dr G. C. Ramsay was placed in charge. Brilliant results followed Dr Ramsay's scientific and practical organisation. I can only summarise them. The health of Europeans and Indians improved, wages and profits increased, 600 young Indians were trained as malaria surveyors, antimalarial work was stimulated throughout India. When war

Ramsay supplied most of the Army for service from West received decorations from His

Majesty the King. Ramsay received the Kaiser's Hind Gold Medal and later the Companionship of the Most Eminent Order of the Indian Empire.

he was made a Companion of the Order of the British Empire (CBE) in 1941. He died in 1942, succeeded by

who, as a lieutenant colonel in the Royal Army Medical Corps, cleared

standing examples  
reduction in the  
tropics—Habana, Panama, Brazil, Northern Rhodesia, India, and  
Malaya. They may be compared to the advantage or disadvantage  
of one another as the critic may be biased. In truth they are com-  
plementary and confirmatory, each has developed on the lines best cal-

unfortunate human beings

Thank God, these six brilliant achievements do not represent the  
total use made of Ross' discovery. In the last 10 years or so there  
has been a great expansion of the work, so that in this Jubilee year

### THE SUN NEVER SETS ON IT

You see it in the southeastern States of the United States of  
America, where the work of the TVA is an outstanding achievement  
in conserving and using water for navigation and agriculture rather  
than running it "down the drain" as a waste product into the sea.

There

Spain

India

lon, where there are schemes to reclaim the land so long abandoned to  
malaria, in Burma, Malaya, the Dutch East Indies; in Borneo, in the  
Philippine Islands where Dr. Paul Russell worked (as well as in India  
and Malaya) before he came to the Far East.

the A

great

Hermes and Gray began as far back as 1911. Their work and experience  
were invaluable in the war, and form the basis of their book on mos-  
quito control which must find a place in every library.

That is the account of our stewardship

### SUNSET

*"Whatever way my years decline,  
I felt and feel, tho' left alone,  
His being working in mine own,  
The footsteps of his life in mine."*

*Section X—TROPICAL VETERINARY MEDICINE*

Dr Herbert Clark Panama—Honorary Chairman  
 Dr I A Galloway United Kingdom—Chairman  
 Dr G D Dhalerao India—Vice Chairman  
 Col Anacleto B Coronel Philippines—Vice Chairman  
 Dr R A. Kelser United States—Secretary  
 Maj T C Jones United States—Assistant Secretary

*Section XI—PUBLIC HEALTH*

Dr L Van Hoof Belgium—Honorary Chairman  
 Dr Angel de la Garza Drito Mexico—Chairman  
 Dr A H Baldwin Australia—Vice Chairman  
 Dr W W Peter United States—Vice Chairman  
 Dr Henry E Meleney United States—Secretary  
 Dr Pasquale Pesare United States—Assistant Secretary  
 Dr Iwao M Moriyama United States Assistant Secretary

*Section XII—MEDICAL AND VETERINARY ENTOMOLOGY*

Dr W D Herm\* United States—Chairman  
 Lt Col Jaswant Singh India—Vice Chairman  
 Dr P A Buxton United Kingdom—Vice Chairman  
 Dr Hector A. Coll Argentina—Vice Chairman  
 Dr Fred C. Bishopp United States—Secretary  
 Dr D F Knippling United States—Assistant Secretary

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*Assistant Secretaries General* Harold G Kissick Dr Willard H Wright  
*Special Assistants* William L Breese Gertrude Henderson J Ward Lowe  
 Maj Jack Walden  
*Protocol Officer* Edward W Nash  
*Press Officer* Frank Standley

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*Archivist* Mary Haslacker  
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*Stenographic Supervisor* Eva A Karpischek





## COOPERATING SCIENTIFIC SOCIETIES

American Academy of Tropical Medicine  
 American Association for the Advancement of Science  
 American Association of Economic Entomologists.  
 American College of Physicians  
 American Dermatological Association  
 American Medical Association.  
 American Public Health Association  
 American Society of Parasitologists.  
 American Society of Tropical Medicine  
 American Veterinary Medical Association  
 Entomological Society of America  
 Medical Society of the District of Columbia  
 National Malaria Society  
 National Research Council  
 Southern Medical Association

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 Dr George K. Strode *Vice Chairman*  
 Mr Clarke L. Willard, *Vice Chairman*<sup>1</sup>  
 Dr Wilbur A. Sawyer, *Executive Secretary*<sup>1</sup>  
 Mr William L. Breese *Secretary*

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Dr Mark F. Boyd <sup>1</sup>	Dr C. F. W. Mnessebeck.
Dr Detlev W. Bronk	Mr Basil O'Connor
Dr W. W. Cort. <sup>1</sup>	Mr W. D. Reed
Dr Rolla E. Dyer <sup>1</sup>	Capt. James J. Sapero <sup>1</sup>
Dr Ernest Carroll Faust <sup>1</sup>	Dr James S. Simmons <sup>1</sup>
Dr John A. Ferrell	Mr Clarence Sterling
Dr Joseph M. Hayman Jr	Dr Lewis H. Weed <sup>1</sup>
Dr William L. Howell	Dr Fred D. Weldman
Dr Raymond A. Kelser	Col. Tom F. Whayne <sup>1</sup>
Mr C. P. Loranz	Dr Louis L. Williams Jr <sup>1</sup>
Dr Thomas T. Mackie	Dr Willard H. Wright <sup>1</sup>

## INTER-SOCIETY COMMITTEE

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 Dr W. W. Cort, *Vice Chairman*<sup>1</sup>  
 Dr Wilbur A. Sawyer, *Executive Secretary*<sup>1</sup>  
 Mr Ernest Gamache *Treasurer*

Dr Mark F. Boyd. <sup>1</sup>	Dr Thomas T. Mackie
Dr Detlev W. Bronk.	Dr Henry E. Meleney
Dr Rolla E. Dyer <sup>1</sup>	Dr C. F. W. Mnessebeck <sup>1</sup>
Dr Ernest Carroll Faust <sup>1</sup>	Mr Basil O'Connor
Dr John A. Ferrell	Mr W. D. Reed <sup>1</sup>
Dr Edward M. Gunn	Dr Fred L. Soper
Dr Joseph M. Hayman Jr	Dr George K. Strode.
Dr William L. Howell <sup>1</sup>	Dr Lewis H. Weed. <sup>1</sup>
Dr Raymond A. Kelser	Dr Fred D. Weldman
Mr C. P. Loranz	

<sup>1</sup> Members of executive committee



Mrs Paul B Magnuson  
 Mrs Robert E Moran  
 Mrs John R Murdock  
 Mrs Thomas B Nolan  
 Dr Elizabeth Parker  
 Mrs Thomas Parran  
 Mrs W W Peter  
 Mrs H L Pugh  
 Mrs W Doyle Reed  
 Mrs Stuart A Rice  
 Mrs William B Sanders  
 Mrs W A Sawyer

Mrs L A Scheele  
 Mrs. Thomas L. Smith  
 Mrs Fred L. Soper  
 Mrs Claude A Swanson  
 Mrs Louis Thomen  
 Miss Helen L Trembley  
 Dr Stella Warner  
 Mrs Tom F Whyne  
 Mrs Clarke L Willard  
 Mrs. Ellen S Woodward  
 Mrs. Willard H Wright

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 Captain Hilton Ross *Vice Chairman*  
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 Mr Dwight Garrison  
 Mr Gale Griswold

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 Dr Hugh S Cumming  
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 Maj Gen Merritte W Ireland

Brig Gen Jefferson R Kean  
 Rear Adm E R Stitt  
 Dr Norman H Topping  
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 Prof G Macdonald, *Secretary*

Prof P A Buxton  
 Mr R W B Dunlop  
 Sir Charles Jeffries

Mr G H Masfield  
 Sir Malcolm Watson  
 Mr A Wigglesworth

#### ROSS CELEBRATION COMMITTEE

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 Dr Wilbur A Sawyer *Secretary*

Dr Fred C Bishopp  
 Capt Otto L Burton M C U S N  
 Brig Gen George R. Callender M C  
 U S A. (Ret)

Dr G Robert Coatney  
 Asst. Surgeon Gen Mark D Hollis  
 U S P H S

FINANCE COMMITTEE<sup>2</sup>

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 Dr George K. Strobe *Vice Chairman*  
 Mr Ernest Gamache *Secretary Treasurer*

Dr Jean Curran                      Dr Edward I Salisbury  
 Dr Thomas T Mackie

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*Convener Section I*  
 Dr R. E Dyer *Program Director*

Dr Fred C Blshopp <i>Convener Section XII</i>	Dr Thomas T Mackie <i>Convener Section VIII</i>
Dr Mark F Boyd <i>Convener Section V</i>	Dr Henry E Meleney <i>Convener Section XI</i>
Dr W W Cort <i>Convener Section VI</i>	Dr John R Paul <i>Convener Section IV</i>
Dr David B Dill <i>Convener Section II</i>	Dr Thomas B Turner <i>Convener Section III</i>
Dr Ernest Carroll Faust <i>Convener Section VII</i>	Dr Fred D Weldman <i>Convener Section IX</i>
Dr John A. Ferrell	Dr Clark H Yeager
Dr Joseph M Hayman Jr	
Dr R. A. Kaiser <i>Convener Section X</i>	

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 Dr Eloise B Cram *Vice Chairman*

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Dr Fred C. Blshopp.	Dr W W Peter
Dr Miguel E. Bustamante	Mrs Stuart A Rice
Dr G Robert Costney	Capt. J J Sapero
Dr James A. Doull	Dr Louis Thomen
Dr William L Howell	Col. Tom F Whayne

## WOMEN'S HOSPITALITY COMMITTEE

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 Dr Eloise B. Cram *Chairman*  
 Mrs. James A. Doull *Secretary*  
 Miss Ruth V Reed, *Liaison with Entertainment Committee*

Dr Sophie D Aberle.	Mrs. Robert O Costney
Mrs. Clinton P Anderson	Mrs. W W Cort.
Mrs. W R. Aykroyd.	Miss Clara E. Councell
Mrs. Marshall C. Balfour	Mrs. R E. Dyer
Mrs. George C. Beach	Mrs. Elisabeth S Enochs.
Mrs. Fred C. Blshopp.	Mrs. E. O Hakansson
Mrs. Raymond W Bilss	Miss Jean Menderson.
Mrs. Frederick L. Brady	Mrs. Rufus Holt.
Mrs. Charles F Brannan	Mrs. William Allen Howard.
Mrs. Miguel E. Bustamante.	Mrs. William L. Howell.
Mrs. T R. Cajigas	Mrs. Clay G Huff.
Mrs. Agnes Chagas.	Mrs. Albert W Kenner

<sup>2</sup> A subcommittee of the intersociety committee.

# RULES OF PROCEDURE

*(As distributed in English, French, and Spanish and adopted by Congresses on May 11, 1948)*

## Section I. CATEGORIES OF MEMBERSHIP

ARTICLE 1 There shall be the following categories of participant the Congresses

- 1 Official delegates Official Government representatives
- 2 Institutional delegates Representatives of invited universal societies and scientific and philanthropic organizations interested tropical medicine
- 3 Members Physicians, scientists, and other professional per-qualified in tropical medicine
- 4 Sustaining members Persons, firms, corporations, and org-zations contributing toward financing the Congresses
- 5 Associates (a) Members of the families of delegates and m-bers, (b) Nonprofessional persons interested in tropical medic
- (c) Students

article 14

Associates shall have the privilege of attending general and sec-meetings but shall not have the right to present papers, partici-in discussions, nor vote

## Section II DUTIES OF ORGANIZING COMMITTEE

- (b) To designate a convener for each section

## Section III PERSONNEL AND DUTIES

ART 3 Temporary President The President of the United St-of America shall designate the Temporary President of the Congre-who shall preside at the opening session and shall continue to pre-

is Congresses the 1  
Credentials  
dents

## BELTSVILLE PROGRAM COMMITTEE

**Dr Fred C. Bishop, Chairman**

Mr J L Boatman  
Dr F P Cullinan  
Dr R E Hodgson  
Mr A S Hoyt  
Mr C A Logan

Dr H C McPhee  
Mr E G Moore  
Dr O E Reed  
Dr C F Speb  
Dr Hazel K Stiebeling

## EXTRA CONGRESS ACTIVITIES COMMITTEE

**Dr James A. Doull, Chairman**

Mr Earl A Dennis  
Mr C Reed Hill

Dr George Payne  
Dr Louis L. Williams, Jr

#### PLENARY SESSIONS COMMITTEE

Mr. Clarke L. Willard, *Chairman*

Dr Wilbur A Sawyer

Dr Willard H Wright.

## SUSTAINING MEMBERS

American and Foreign Power Co Inc  
American Medical Association  
American National Red Cross  
Arabian American Oil Co  
Burrroughs, Wellcome & Co  
California Texas Oil Co  
Cinchona Products Institute  
Coca Cola Export Corp  
International Business Machines Corp  
Lederle Laboratories  
Pan American World Airways  
Schering Corp  
Socony Vacuum Oil Co, New York, N Y  
Standard Oil Co of New Jersey  
United Fruit Co  
Youngstown Sheet & Tube Co

#### OTHER CONTRIBUTORS

The above private organizations have contributed to the Intersociety Committee toward the expenses of the Congresses

(g) To perform such other functions as may be assigned to him by the rules of procedure, by the Congresses or by the President of the Congresses

ART 9 Section officers The sections shall each elect a chairman and two vice chairmen The convener shall become Secretary

#### *Section IV CREDENTIALS AND RESOLUTIONS COMMITTEES*

ART 10 (a) A Committee on Credentials shall be appointed by the Temporary President and shall be composed of one member from each of three of the official delegations The committee will examine and report to the Congresses on the credentials presented

(b) A Committee on Resolutions shall be appointed by the President The committee will review resolutions and proposals to be submitted in general meetings and decide whether and in what form these shall be submitted to consideration

#### *Section V SECTIONS*

ART 11 The Congresses shall be composed of the following sections

- I Research and Teaching Institutes
- II Tropical Climatology and Physiology
- III Bacterial and Spirochetal Diseases
- IV Virus and Rickettsial Diseases
- V Malaria
- VI Helminthic Diseases
- VII Protozoan Diseases
- VIII Nutritional Diseases of the Tropics
- IX Tropical Dermatology and Mycology
- X Tropical Veterinary Medicine
- XI Public Health
- XII Medical and Veterinary Entomology

No sections other than those enumerated above shall be recognized The various sections of the Congresses may meet simultaneously The details of arrangements of the program of each section will be in charge of the Program Committee of the Organizing Committee and the respective conveners, with the collaboration and approval of the Secretary General

#### *Section VI LANGUAGES*

ART. 12 The official languages of the Congresses shall be (1) English, (2) French, and (3) Spanish English shall be used as the working language in the conduct of the deliberations and the drafting of the conclusions of the Congresses However, discussions from the floor may be conducted in any of the three languages

It is permissible to speak in other languages if the speaker furnishes interpretation into an official language

The Permanent President shall be elected by the Congresses

The Congresses shall also elect three Vice Presidents who in the absence of the President shall preside in rotation in alphabetical order by country in English

ART 6 The duties of the Permanent President shall be

(a) To appoint a Committee on Resolutions as provided in article 10 (b)

(b) To preside at the meetings of the Congresses (He may delegate the Chair)

(c) To concede the floor in the order in which requested

(d) To decide all questions of order raised during the debates of the Congresses Nevertheless, should any delegate or member so request, the ruling made by the Chair shall be submitted to the Congresses for decision by a majority vote

(e) To call for votes and to announce the result of each vote to the Congresses

(f) To determine the order of business

(g) To prescribe all necessary measures for the maintenance of order and compliance with the rules of procedure

ART 7 Honorary Presidents and Vice Presidents

The Congresses may elect three Honorary Presidents and six Honorary Vice Presidents on the nomination of the Organizing Committee and each section may elect an Honorary Chairman on nomination from the floor

ART 8 Secretary General and Deputy Secretary General

The Secretary General and the Deputy Secretary General of the Congresses shall be appointed by the President of the United States

The duties of the Secretary General and Deputy Secretary General shall be

(a) To organize, direct, and coordinate the work of the secretaries, assistant secretaries, secretaries of committees, interpreters, clerks, and other employees whom the Government of the United States of America may appoint for service with the secretariat of the Congresses Both shall also assist in and coordinate the work of the sections and committees of the Congresses

(b) To serve as the principal adviser to the President of the Congresses on parliamentary, procedural, and protocol matters

(c) To receive, distribute, and answer the official correspondence of the Congresses in conformity with the resolutions of that body

(d) To prepare or cause to be prepared under his supervision, the minutes of the

on which they are required to present reports, and place at the disposal of the committees and sections everything that may be necessary for the discharge of their duties.

(f) To prepare and circulate notices of the hour and place of meetings



résumé should be in one of the official languages and should not exceed 500 words

The chairman of any session may give the floor to persons not delegates or members but who are particularly qualified to discuss the subject under consideration

### *Section XI* MOTIONS, RESOLUTIONS, RECOMMENDATIONS, ETC

ART 17 (a) All motions, resolutions, and recommendations shall be presented in one of the official languages

(b) If it is desired to offer a motion that applies to a question not appearing on the agenda, it must be presented in writing to the chairman of the section or to the Secretary General

(c) All resolutions pertaining to the agenda of any of the various sections shall be presented in writing to the chairman of that section.

(d) It is the duty of the secretary of each section to prepare recommendations, resolutions, or conclusions of the discussions pertaining to the work of the section

(e) The presentation of any resolution shall not exceed 5 minutes and the discussion by any one member shall not exceed 3 minutes

(f) All resolutions to be presented in plenary sessions shall be submitted in writing to the Secretary General for reference to the Resolutions Committee, which Committee shall make report thereon to the Congresses

(g) The resolutions of the Congresses shall be acted upon in a plenary session of the Congresses and decided by majority vote

### *Section XII* APPROVAL OF AND AMENDMENTS TO THE RULES

ART 18 These provisional rules shall be approved in plenary session of the Congresses and shall be subject to subsequent modification only by a vote of two thirds of the Congresses

### *Section XIII* REPORT OF PROCEEDINGS

ART 19 After adjournment, a report of the proceedings of these Congresses will be printed and forwarded gratis to all delegates and members, and to those associate members who have paid the prescribed special fee upon registration

## RULES OF PROCEDURE

### Section VII PAPERS

ART 13 The Organizing Committee shall issue invitations for papers. In general, the following regulations shall govern the submission of papers

- (a) Each paper shall be accompanied by an abstract of not more than 300 words
  - (b) Papers shall be limited to 3 000 words, and the time of presentation to 20 minutes
  - (c) All papers shall be typewritten
  - (d) In order to facilitate the work of the officers of the several sessions, the abstracts shall be in the hands of the Organizing Committee not later than February 29, 1948 and copies of the papers, not later than March 31, 1948
  - (e) Papers and abstracts should be submitted in one of the three official languages
  - (f) Authors who may be desirous of revising their papers subsequent to the Congresses, must submit these revised papers not later than 10 days after the conclusion of the Congresses
  - (g) Papers may be accompanied by illustrations and tabular material for purposes of clarification. It is suggested that illustrations be limited in number
  - (h) In view of the desire to take full advantage of the great progress of recent years the papers submitted should have special reference to the trend of recent development in the subjects concerned
- In the event that the abstracts are received in sufficient time an effort will be made to duplicate and distribute them during the Congresses

### Section VIII VOTING

ART 14 All delegates and members shall have the privilege of voting on such matters as require a decision of the entire Congresses, except on a question of organization, in which case each country shall have one vote only. Decisions will be taken by majority vote

### Section IX QUORUM

ART 15 A majority of the states participating shall constitute a quorum in plenary sessions. The sections shall determine their own quorum

### Section X DISCUSSIONS

ART 16 No one may speak from the floor for more than 3 minutes. One speaker may speak more than once in the discussion of a paper or subject unless the presiding officer gives him permission to do so. Speakers are requested to hand a written résumé of their remarks to the Secretary General or to the Secretary of the section. The

9 a. m.—6 p. m.

Scientific and Commercial Exhibits, Hall of Nations, Washington Hotel

10 a. m.—4 p. m.

Motion Pictures, Room 43, National Museum

9 30 a. m.—12 m.—SECTION MEETINGS

Section III—Bacterial and Spirochetal Diseases Session 2—Syphilis, Yaws, and Pinta Departmental Auditorium, Main Hall

Section IV—Virus and Rickettsial Diseases Session 2—The Rickettsial Diseases Auditorium of National Museum

Section VII—Protozoan Diseases. Session 1—Amebiasis. Department of Commerce Auditorium

Section VIII—Nutritional Diseases in the Tropics Session 1—Background Problems of Nutrition in the Tropics Departmental Auditorium, Room B

12 30 p. m.

Special Luncheon by American Foundation for Tropical Medicine Hotel Statler

2-4 30 p. m.—SECTION MEETINGS

Section III—Bacterial and Spirochetal Diseases Session 3—Plague Department of Commerce Auditorium

Section IV—Virus and Rickettsial Diseases Session 3—Infectious Hepatitis Departmental Auditorium, Room B

Section VIII—Nutritional Diseases in the Tropics Session 2—Nutritional Deficiencies and Problems of Special Areas in the Tropics Auditorium of National Museum

Section XII—Medical and Veterinary Entomology Session 1—Mosquitoes and Disease Departmental Auditorium, Main Hall

5-7 p. m.

Hospitality Session, Shoreham Hotel

6 30 p. m.

Dinner Meeting of Experts on Plague Shoreham Hotel

8-10 p. m.—SECTION MEETINGS

Section I—Research and Teaching Institutes Session 1—Research and Teaching in Tropical Medicine Departmental Auditorium Main Hall (Joint Session with Section XI)

Section II—Tropical Climatology and Physiology Session 1—Tropical Climatology and Physiology Departmental Auditorium Room B

## GENERAL PROGRAM

SUNDAY, MAY 9

11 a. m.-5 p. m.

Registration at Washington Hotel, Pennsylvania Avenue and Fifteenth Street NW

8-9 30 p. m.

Joint meeting of Organizing and Intersociety Committees, Room 1122, Division of International Conferences, Department of State

MONDAY, MAY 10

9 a. m.-2 p. m.

Registration and Information at Departmental Auditorium Foyer

3-5 p. m.

Registration and Information at Washington Hotel

9 a. m.-6 p. m.

Scientific and Commercial Exhibits, Hall of Nations, Washington Hotel

2-4 p. m.

Motion Pictures Room 43, National Museum

11 a. m.-1 p. m.

Opening Plenary Session, Departmental Auditorium, Main Hall

2-4 30 p. m.—SECTION MEETINGS

Section III—Bacterial and Spirochetal Diseases Session 1—Tuberculosis Department of Commerce Auditorium

Section IV—Virus and Rickettsial Diseases Session 1—Viruses in General Auditorium of National Museum.

Section V—Malaria Session 1—Parasite Host Relationship Departmental Auditorium, Main Hall

Section XI—Public Health Session 1—Education and Research Departmental Auditorium, Room B (Joint Session with Section I)

5-7 p. m.

Official Reception, Pan American Union

Music by United States Marine Corps Band

TUESDAY, MAY 11

9 a. m.-5 p. m.

Registration and Information at Washington Hotel

## 2-4 30 p m —SECTION MEETINGS

Section V—Malaria Session 2—Entomology Departmental Auditorium, Main Hall

Section VII—Protozoan Diseases Session 2—The Blood and Tissue Flagellates Department of Commerce Auditorium

Section IX—Tropical Dermatology and Mycology Session 2—Tropical Dermatoses Auditorium of National Museum

Section X—Tropical Veterinary Medicine Session 2—Foot and Mouth Disease, Schistosomiasis, Epizootic Lymphangitis, Anaplasmosis and Salmonella Infections Departmental Auditorium, Room B

5 p m.

Tea by Mrs Truman at the White House for wives of Members and Delegates

5-7 p m.

Reception by the Health and Sanitation Division, Institute of Inter American Affairs, for gentlemen delegates and visitors from Latin America Hotel Mayflower

## FRIDAY, MAY 14

9 a m-5 p m.

Registration and Information at Washington Hotel

9 a m-6 p m

Scientific and Commercial Exhibits, Hall of Nations, Washington Hotel

10 a. m-4 p m.

Motion Pictures, Room 43, National Museum

## 9 30 a m-12 m —SECTION MEETINGS

Section III—Bacterial and Spirochetal Diseases Session 4—Enteric Diseases, Cholera, Electron Microscopy Departmental Auditorium, Room B

Section IV—Virus and Rickettsial Diseases Session 4—Yellow Fever, Dengue, and Sandfly Fever Auditorium of National Museum

Section V—Malaria Session 3—Chemotherapy Departmental Auditorium, Main Hall

Section XII—Medical and Veterinary Entomology Session 2—Flies and Disease Department of Commerce Auditorium

1 p m

Luncheon for the Ladies, Army Navy Country Club, Arlington

12 30-1 45 p m.

Special Luncheon for members of the Royal Society of Tropical Medicine and Hygiene, Hotel Washington

GENERAL PROGRAM

WEDNESDAY, MAY 12

9 a. m.-5 p. m.

Registration and Information at Washington Hotel

9 a. m.-6 p. m.

Scientific and Commercial Exhibits, Hall of Nations, Washington Hotel

10 a. m.-4 p. m.

Motion Pictures, Room 43, National Museum

2 30 a. m.-4 30 p. m.

Visits to Agricultural Research Center, United States Department of Agriculture, Beltsville, Md Demonstrations Luncheon at Beltsville

8 30-10 p. m.

Exercises to Commemorate the Establishment by Walter Reed of the Mosquito Transmission of Yellow Fever Departmental Auditorium Main Hall

9 a. m.-5 p. m.

THURSDAY, MAY 13

Registration and Information at Washington Hotel

9 a. m.-6 p. m.

Scientific and Commercial Exhibits Hall of Nations Washington Hotel

10 a. m.-12 m.

Special Meeting of Malaria Experts Conference Room Division of International Conferences Department of State

10 a. m.-4 p. m.

Motion Pictures, Room 43, National Museum

3 30 a. m.-12 m.—SECTION MEETINGS

Section VI—Helminthic Diseases Session 1—Filaria and other Helminthic Diseases. Auditorium of National Museum

Section IX—Tropical Dermatology and Mycology Session 1—Mycology Departmental Auditorium, Room B

Section X—Tropical Veterinary Medicine Session 1—Trypanosomiasis Rinderpest and Newcastle Disease Departmental Auditorium Main Hall

Section XI—Public Health Session 2—Health and Medical Services in the Tropics Department of Commerce Auditorium

4-1 30 p. m.

Luncheon of the American Academy of Tropical Medicine, Washington Hotel

## SUNDAY, MAY 16

10 a m-2 p m

Visit to Mount Vernon Ceremony at the Tomb of Washington  
 Trip by boat (Busses to wharf at 9 30 a m)

## MONDAY, MAY 17

9 a m-5 p m

Registration and Information at Washington Hotel

9 30 a m-12 m—SECTION MEETINGS

Section III—Bacterial and Spirochetal Diseases Session 6—  
 Leprosy Auditorium of National Museum

Section IV—Virus and Rickettsial Diseases Session 6—Arthro-  
 pod borne Encephalitides and Rabies Department of Commerce  
 Auditorium.

Section V—Malaria Session 6—Present Proportions of the  
 Global Malaria Problem Departmental Auditorium, Main Hall

Section XI—Public Health Session 4—Public Health and Vital  
 Statistics Problems Departmental Auditorium, Room B

1 30-5 p m

Visits to the National Institute of Health and the Naval Medical  
 Research Institute in Bethesda, and to the Army Medical Department  
 Research and Graduate School, Washington

7-10 30 p m

Dinner for Delegates, Members, and Associates Mayflower Hotel

## TUESDAY, MAY 18

9 a. m-5 p m

Registration and Information at Washington Hotel.

9 30 a m-12 m

Visits to scientific institutions in or near Washington

9 30 a m-12 m

Visit to Johns Hopkins University School of Hygiene and Public  
 Health

2 15-3 p m

Closing Plenary Session Departmental Auditorium, Main Hall

# GENERAL PROGRAM

## 2-4 30 p m—SECTION MEETINGS

Section III—Bacterial and Spirochetal Diseases Session 5—L  
tospiriosis, Effect of Environment Departmental Auditorium, Ro  
B

Section V—Malaria Session 4—Immunity, Malaria Control D  
partmental Auditorium, Main Hall

Section VI—Helminthic Diseases Session 2—Schistosomiasis and  
other Helminthic Diseases Department of Commerce Auditorium  
Section XII—Medical and Veterinary Entomology Session 3—  
Ticks, Mites, Lice and Fleas. Auditorium of National Museum

8 30-10 p m.

Exercises to Commemorate the Fiftieth Anniversary of the Dis  
covery by Ross of the Method of Transmission of Malaria Depart  
mental Auditorium, Main Hall

SATURDAY, MAY 15

9 a m-2 p m.

Registration and Information at Washington Hotel

9 a m-2 p m

Scientific and Commercial Exhibits Hall of Nations, Washington  
Hotel

10 a m-4 p m.

Motion Pictures, Room 43, National Museum

9 30 a m-12 m.—SECTION MEETINGS

Section IV—Virus and Rickettsial Diseases Session 5—Tropical  
Poliomyelitis. Departmental Auditorium, Room B

Section V—Malaria Session 5—Malaria Control Departmental  
Auditorium, Main Hall

Section XI—Public Health Session 3—The Tuberculosis Problem  
in the Tropics. Department of Commerce Auditorium

Section XII—Medical and Veterinary Entomology Session 4—  
Fratoma, Insecticides, Toxicology and Equipment Auditorium of  
the National Museum.

4 p m

Special Meeting of persons interested in Schistosomiasis. Con  
ference Room, Division of International Conferences, Department of  
te.

p m.

Children Party at Dumbarton Oaks for Delegates, Members As  
sistants, and Ladies, under auspices of Harvard School of Public  
Health.





# PROGRAM OF SECTIONS

## FACILITIES FOR RESEARCH AND TEACHING IN TROPICAL MEDICINE IN AFRICA

A I MANAFFY, *Director of Colonial Medical Research, Colonial Office, London Staff Member, International Health Division of the Rockefeller Foundation, 1923-46 Member, West African Yellow Fever Commission, 1925-34 Director, Yellow Fever Research Institute, Entebbe, Uganda, 1936-46*

The important part which research in tropical medicine has played and must continue to play in the development of the African continent is well recognized, and it is appropriate that we should consider at this time some of the facilities which have been provided for carrying it out. Also, since the first requisite of any program of research is an adequate number of trained individuals, the discussion should embrace an examination of the steps which have been taken in Africa to provide institutions where students who are interested in tropical medicine can receive instruction. But before proceeding further, it might be well to clarify the sense in which it is intended to employ the term "research." It will be appreciated that in large areas of the African continent conditions differ in many respects from those existing in the more developed countries. In such countries the modern tendency is to think of medical research as comprising highly specialized investigations which require the aid of precise scientific technique. It is proposed here to use the term in a wider sense and to include in it all investigations designed to discover knowledge applicable to the maintenance of health or to the causation, prevention, and cure of disease. It will not, however, include routine activities in the laboratory or elsewhere, for although such activities may often result in observations which might be used to initiate research, it is felt that they should not themselves be placed in that category.

It will not be possible, of course, in the time at my disposal to

the systems which have been adopted by the various administrations to meet their particular research problems. Finally, the coordination

opportunities for research. However, in places so diverse in regard

## SECTION I

### Research and Teaching Institutes

#### Session 1. RESEARCH AND TRAINING IN TROPICAL MEDICINE

(JOINT SESSION WITH SECTION XI, PUBLIC HEALTH)

*Tuesday, May 11—8 15-10 30 p m*  
*Departmental Auditorium, Main Hall*

The meeting was convened by Dr Wilbur A Sawyer, who conducted the nomination and election of an honorary chairman, a chairman, and two vice chairmen. The list of officers of section I, thus completed, was as follows

- Dr P Morales Otero, Puerto Rico, honorary chairman.
- Dr B G Macgraith, United Kingdom, chairman
- Dr Geraldo de Paula Souza, Brazil, vice chairman
- Dr Kenneth Mellanby, Nigeria, vice chairman
- Dr Wilbur A Sawyer, United States, secretary
- Maj Jack T Walden, United States, assistant secretary

Dr Macgraith presided while the papers of section I were presented and discussed

Cullinan (3), consulting physician, East Africa Command, during the recent war, made a plea for the encouragement of this type of effort and stressed the "need for widespread coordinated epidemiological and clinical observation of basic medical problems."

Medical research in Africa has by no means been confined to the permanent local agencies already referred to. It has frequently happened that individual territories have provided facilities for undertaking the investigation of special ad hoc problems. Examples of

nutrition in Nyasaland and the Gambia; on plague, relapsing fever, and typhus in Kenya; on typhus in Tunisia; and on yellow fever in the French possessions in West Africa.

And now we come to an important contribution consisting of a . . . en sponsored by . . . with individual . . . zed by the health . . . 27 in connection . . . and the studies in yellow fever which are being carried out by the International Health Division of the Rockefeller Foundation in tropical Africa, in co operation with local governments, are examples of the efforts of international agencies. In addition, one thinks of the numerous French and Belgian missions and permanent organizations, like the Belgian Foreams, which have been sent to . . . specific problems, of the work of . . . Medicine at the Sir Alfred Jones L . . . studies carried out by members of the teaching staff of the London

Bureau maintains an international outlook, and no . . . The of any importance, regardless of its source, escapes its notice.

# 1 RESEARCH AND TEACHING INSTITUTES

size, population, and particularly resources, such as exist in Africa. It is obvious that the extent to which the different countries have developed every stage possible to provide facilities for research varies widely. The surprising, then, to find that these facilities include well-equipped Medical Research Institute with a large staff of full time workers, to the small laboratory with 2 or 3 rooms staffed by a pathologist and one assistant. This institute in Johannesburg is an outstanding example of the former. The total staff employed is increased in size in recent years and has established branches in Port Elizabeth, Bloemfontein, and Rietfontein. The annual report of the Institute by officers of the institute are listed in the annual report of the director, and its perusal provides ample evidence of the great volume of work done, as well as of the wide variety of the problems which are studied. Other examples of the larger type of institute are to be found in the Public Health Laboratories in Cairo, the Pasteur Institute in Dakar (2); the State laboratory in Leopoldville, the Stack Laboratories in Khartoum, and the Medical Research Laboratory in Nairobi, all of which provide liberal accommodation for research workers. The smaller type of laboratory is found, as would be expected, in the smaller dependencies in tropical Africa. The technical staff of the laboratory in Freetown, Sierra Leone, for example, comprises one pathologist and one medical entomologist, while that in Zomba, Nyasaland, is limited to one pathologist. Finally there are many laboratories which might be classed as intermediate in size and in all of which accommodation and equipment are available for research workers.

From this very brief survey, it is evident that the facilities, in the form of buildings and equipment, which have been provided for medical research work in many countries in Africa, although by no means adequate, are in fact, considerable. It should be borne in mind, however, that in the smaller laboratories, and, indeed, in many of the larger ones, the staff is responsible for an immense amount of routine work with the frequency with which he was told that the staff was bound to suffer, and it too frequently happens that it is laid out altogether or is left to the enthusiast who does what he can in spare time. Nevertheless, despite the difficulties, the amount of work which has been accomplished over the years is by no means negligible, and this can be readily verified by anyone who takes the trouble to peruse the literature.

At no point mention should be made of, and due credit given to, the accomplishments of a number of individuals who were not recruited for laboratory or research work. It would be impossible to quote many instances in which observations and original work by such persons have had results of great practical importance.

present, is lamentably small in relation to the extent of the population which they are intended to serve. To provide anything approaching an adequate staff, many more and much larger schools would be necessary, but this will take time, since it cannot be brought about until there is a corresponding increase in the facilities for pre vocational training. A medical school cannot function effectively in a community in which there is not an adequately educated section of society from which to draw its recruits.

It is quite apparent, then, that so far as tropical medicine in Africa is concerned, there is, as yet, no local source of persons whose training and experience fit them to take a leading part in the medical research problems of that vast area. The workers who are urgently needed to undertake the investigation of these problems must, for many years to come, be found from sources outside Africa. This means that in the future, as in the past, the responsibility for recruiting and training the necessary research staff will fall mainly upon the schools and research institutions of those European countries having dependencies in Africa. If this is to be accomplished successfully, there is much still to be done. It is true that the recent World War provided a powerful stimulus to research which was productive of much valuable new knowledge. It is equally true that

disease, are still sadly deficient. These institutions must be rehabilitated and strengthened with the minimum of delay and, where necessary, new centers should be created in order that the flow of trained staff may again become adequate to meet the need.

There remains for consideration the important question of the coordination of research in Africa. A survey of the facilities that

permanent research workers employed in Africa and that a few studies which have been made have been carried out by special missions sent out from the home country. A very considerable degree of coordination has been achieved at the Pasteur Institute, which has branches in Africa.

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her hand,

value of such publications to the research worker in Africa, cut off as he so often is from library facilities, is too obvious to need further comment.

Next let us look briefly at the institutions in Africa which provide instruction in tropical medicine. At the outset it can be said that there are no training centers in Africa comparable with the schools of tropical medicine in Europe, which specialize in postgraduate training and which have a full time staff engaged in teaching and research. There are three medical schools in the Union of South

made available a diploma course for graduates, for whom it is especially able to cater because of its close association with the South African Institute for Medical Research.

for this purpose. In most of the colonial territories, the medical services were originally concerned mainly with the care of the immigrant white population. These services were gradually enlarged to

attended by an ever increasing need for African assistants, and facilities were provided for training a rapidly growing body of subordinate staff, such as dispensers, nurses, laboratory technicians, sanitary inspectors, dressers, and midwives. As time went on, it became evident that there was need for a more highly trained auxiliary staff to assist with the increasing volume of routine diagnosis, preventive measures, and treatment.

To provide these medical assistants, special schools were necessary,

of training is now usually of 6 years' duration and, in general, it follows that given in medical schools in Europe, with minor modifications to meet local conditions. The quality of the teaching is good, and some schools are already looking forward to the time when they will be equipped to train African practitioners who will be fully qualified in the sense in which we recognize that term. Unfortunately the number of students who have received this training up to the



organized from time to time to discuss problems of common interest. Discussions of this kind are undoubtedly of great value, and every effort should be made to encourage them and to arrange for them to be held at more frequent intervals, but, in my view, it is doubtful if

of interests in one or more central research institutions is urgently necessary." He believes, for example, that "A research institution on trypanosomiasis and tsetse flies serving most of the African territories, and situated in Central Africa, should be supported by them all."

Whatever the answer may be, there can be no doubt that tropical disease in Africa presents many problems, the solution of which would be greatly facilitated if some arrangement could be made whereby investigators would no longer be hampered by artificial political boundaries. There is a tendency in Africa for the authorities in one territory, when confronted by an outbreak of a dangerous and notifiable disease, to lay the blame for its introduction on a neighboring territory. For example, there was a time in West Africa when the British got their yellow fever from the French and vice versa, but this situation no longer exists since it is now generally recognized, as the result of recent work, that the disease is endemic throughout the whole area. The concept of international research institutions is one which deserves careful consideration and one which should not be lightly dismissed as being impossible of accomplishment. In this connection it is encouraging to find that at the British Commonwealth Scientific Official Conference in London in 1946, it was agreed that a

where, knows no boundaries, and there might well be extended to the international field the motto of the Royal Society of Tropical Medicine and Hygiene "Zonae Torridae Tutamen."

## REFERENCES

- (1) CLUVER, E. H. Medical Research in the Union of South Africa. Presented at the Royal Society Empire Scientific Conference London 1946.
- (2) MATHIS, C. L'Oeuvre des Pasteuriens en Afrique Noire Afrique Occidentale Française. Presses Universitaires de France Paris, 1946.
- (3) CULLINAN, E. R. *Tr. Roy Soc Trop Med & Hyg* 39: 353 1946.
- (4) LEAGUE OF NATIONS HEALTH ORGANIZATION. Final Report of the International Commission on Human Trypanosomiasis 1928.
- (5) RESEARCH NOTES *J Parasitol* 33: 283 1947.
- (6) TROLLI, G. Bruxelles—méd 20 Nos 7 8 9 10 1939-40.

selection of problems for study has been left largely to individual discretion, and there has been no machinery to direct efforts toward the

economical and advantageous use of existing knowledge and experience and that it will lead to the best employment of the limited staff which is available. It will make free interchange of staff possible, will avoid overlapping, which undoubtedly has occurred in the past, and will go far to secure continuity in research. In 1945 a committee, whose members were chosen because of their expert knowledge of the various branches of tropical medicine, was set up to advise the Secretary of State for the Colonies on all matters pertaining to medical research, and a Director of Colonial Medical Research has been ap-

other sources. Research projects will be selected in collaboration with local authorities in the colonies, including those in Africa, and will be financed in part from central research funds and in part by grants made by the particular colony or colonies where the work is to be done. It is hoped that this new organization, the details of which will be announced shortly, will provide the machinery necessary to ensure the proper coordination of medical research throughout the

perment, and to study the ecology of man in his total environment. The Forearm (6) organization of the Belgian Congo began such a study of the health units defined the study

In the sleeping (particularly the great, as well as the medical, the populations on new

ground have been faced in the spirit of research. Extensive survey studies of the Africans in East Africa are now being planned as a research undertaking.

The important problem of the coordination of research in Africa on an international basis is a more difficult one. In the past this has been mainly dependent on international conferences which have been

are engaged in tropical problems will come. In the permanent buildings, which we hope to start next year, there will be a very extensive selection of laboratories specifically intended for visiting workers. In addition to that, we hope to have accommodations in the halls of residence for senior members where they can live while studying problems of the country. We hope to provide facilities so that people can come for short times and not be faced with the difficult problem of staff and the domestic questions which so often confront visitors in the Tropics. I hope that people from other countries, as well as from the British Empire, will make use of these opportunities.

- (7) KARK, S L. South African M J 16 197, 1942, South African M J 18 39 1944.
- (8) McLETCHIE J L. Farm and Forest, Ibadan, Nigeria 6 69 1946.
- (9) SHONLAND, B F J. Africa as a Regional Area for Fundamental Research. Presented at the Royal Society Empire Scientific Conference, London, 1946.

### ABSTRACT OF DISCUSSION

brief consideration of the new organizations, and particularly the various university colleges, which are being set up at the present time in different parts of the British Commonwealth.

of African medical students to proceed through the whole of the medical course and obtain a qualification which is recognized as equal to that from medical schools in temperate countries. At first, we shall only be able to deal with a comparatively small entry, perhaps about 20 students per year. As Dr Mahaffy explained, the backwardness of secondary education is such that it will be difficult for some time to

that number of places in the near future

highly qualified staff, not only from Britain and the British Commonwealth, but from other countries as well. We have already made a start in that way, in fact we have appointed one Danish professor in the arts faculty, and we hope that other chairs and lectureships will be filled from other countries. As the medical school develops, it is hoped that it will also be a place in which post graduate instruction may be given, both to indigenous people and also to those from other countries.

be able to take an active part in research. Also, it is hoped that Ibadan will become a center to which medical research workers who

pecially the Haffkine plague vaccine and sera. Its research has been largely on plague, typhus, and pharmacology.

The King Institute of Preventive Medicine, located in a suburb of Madras, has a reputation for extensive production of biologics, including a large vaccine lymph output. Besides diagnostic services and biologic production, it is actively interested in virus and rickettsial studies. The Public Health Section has studied water and sewage

which has

into two parts. There was an extraordinarily rapid division of territory, funds, property, and personnel between the two dominions. As a result of geographic and climatic fields, the Dominion is faced now with the need of creating new laboratories and institutions.

### *Ceylon.*

former British colony has also achieved dominion status very recently. Since the pandemic of 1934-35 the Government of Ceylon has been fully conscious of its malaria problem. The local epidemiology of this disease has been investigated carefully. The Department of Malaria Control and the Government Medical Entomologist have a highly developed system of malaria and anopheles measurements, malaria control has been gradually extended. With the advent of DDT, an island wide program has been organized, and 30 to 40 percent of the 7,000,000

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tute, an

Bacteriology, parasitology, and nutrition are the major divisions of this institute, which was recently renamed the Malaria Research Institute of Ceylon. Studies of nutrition have been a major interest in Ceylon. Teaching requirements aside from the undergraduate medical curriculum have been met in Ceylon by special courses for public health and malaria workers, largely on a basis of lectures, laboratory, and practice in the field.

*Japan*—In Japan public health and medical services are being re-established and reorganized on a civilian basis. These activities are pursued through Japanese governmental and educational agencies with the advice and directives when necessary of the Public Health and Welfare Section of SCAP (Supreme Commander for the Allied Powers). Two prewar with tropical disease problems infectious Diseases and the Institute did not suffer major damage but staffs and equipment were demoralized and depleted by the war. Reestablishment of their activities is in progress. The School of Public Health has resumed its training of

# RESEARCH AND TEACHING IN TROPICAL MEDICINE IN THE FAR EAST

M C BALFOUR, M D, *Regional Director in the Far East, International Health Division of the Rockefeller Foundation*

The chairman of the Program Committee of the Congresses on Tropical Medicine and Malaria in Washington has requested that I review recent developments in the Far East in the study of tropical

better acquainted with the subject will amend or amplify my limited

Developments in research and teaching of tropical medicine since the last Congress in 1938 will be reviewed by countries. Stress will be laid on developments during and following the recent war. The principal institutions identified with research and teaching to be cited

information has not been available

In strict terms the topic can be covered very briefly. The only formal course of graduate teaching given in this field was one at the Calcutta School of Tropical Medicine. Fundamental research in tropical or epidemic diseases has been severely limited in the Far East during the war period.

Almost all developments and accomplishments have been of an applied or practical nature, related to the war efforts of the different countries. Most of the countries of the Far East, excluding only India and Ceylon, were under occupation for three or more years, parts of China were occupied for 8 years. The isolation and economic

ditions have in some ways accelerated the treatment and prophylaxis of tropical diseases. Because by necessity attention was focused on food, most countries in the Far East have augmented their nutritional investigations and services.

*China*—China's interest in tropical medicine cannot be separated readily from its interest in public health and medical study and teaching. Aside from the medical colleges of China, the National Institute of Health and its branches are the principal institutions of research

We in India, and I am sure elsewhere, are fully alive to the situation. Comprehensive plans have been drawn up which call for the upgrading of our existing medical schools and the organization of new ones on modern lines. The fulfillment of these plans in India and the Far East are hampered and delayed for lack of means. We don't lack the necessary talent, but we must develop our physical resources to permit of an advance on the scientific road. We must organize and provide adequate facilities for our workers. For such a purpose, world resources should be pooled and harnessed to common good.

Before closing, I must take note of Dr. Balfour's reference to the human population problem. It is indeed a vital problem, which is exercising the best minds. The disproportion between food production and the increase in human population is becoming more alarming every day. We must make it a central problem of study to meet it. But I should like to say at this stage that in poor countries like India and the Far East, to stabilize the situation it is essential that we do raise the dignity of human life. We must rapidly develop our resources to make a decent physical and cultural life possible for all, and thus make it worth emulating in the minds of men. Ultimately, the solution must come from the inner conversion of men.

health officers and public health nurses with a new orientation. Within the Institute of Infectious Diseases a National Institute of Health has newly been created. Its functions will include the standardization and control of biologics.

Under the stimulus and supervision of the Public Health and Welfare Section, an outstanding accomplishment has been a series of nutrition surveys, conducted during the postwar period. These surveys have been made quarterly, of an extensive and representative sample of urban and rural populations. Their purpose has been to record actual food consumption and to measure the health or nutri-

a part of the prospective Malayan Union, it will be recalled that the Institute for Medical Research has made notable contributions, particularly in the field of malaria. Under occupation by Japan, the institute at Kuala Lumpur carried on with its local staff while most of the British officers were interned.

Fortunately there was no major loss of equipment or library. The institute has resumed its previous organization, including the divisions of bacteriology, chemistry, pathology, entomology, malaria research, and nutrition. Virus and rickettsial diseases, nutritional surveys, and malaria field studies on the use of the synthetic drugs are

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tropical medical problems. That the Medical Research Institute of Kuala Lumpur will continue to make sound scientific contributions can be confidently anticipated.

*Republic of the Philippines*—The Philippine Republic is also struggling valiantly to rehabilitate its institutions and services after the overwhelming damage which occurred during reoccupation. Science and teaching have suffered the most, although teaching and

and teaching. It is estimated that 60 percent of the city of Manila was destroyed. The university center gives an impression of even greater destruction. However, in buildings which appear largely demolished, one finds that laboratories and classrooms are still in action. The Bureau of Science is practically a total loss, while the

graduate teaching has not yet been resumed.



In the days of its infancy my country, like all other infants, continually cried for sustenance both material and intellectual to those responsible for its birth. At times the grim specter of famine was the dominant feature of the settlers' lives. Medical men were few, yet strangely enough, often of surprising merit, though this quality was not always exercised in the pursuit of their profession. As an

sea, discovered coral reefs, and partially played a part in the hero of an adventure story for boys.

The explorations of Bass were merely a transmutation of the research spirit in a doctor, the search for truth and knowledge.

At this point, we leave what many would consider the most interesting period of our history, with the sole piece of medical research accomplished, namely, that it was possible for settlers to maintain themselves in this strange land.

In our next period, 1820-50, which can be likened to childhood, we find that the work consisted largely of setting bones and mending broken heads, when 40 miles on horseback over rough bush tracks to attend a patient

such men having time to carry out research? In circumstances such as these, the ordinary ills of childhood and of the family were treated by the mother of the household, who brought to this task a knowledge of herbs and simples for the common ailments of man. Unfortunately, much of this knowledge, acquired in other lands was of little use in the land of their adoption where they were faced with a completely strange flora. Undaunted, they tried these new plants as remedies, and partly by trial and error and partly on aboriginal advice they succeeded in their ministrations. Surely these were the research workers of this period. The doctor's wife, in the numerous absences of her husband, was placed in the difficult position of having

development of any pioneering country so greatly depends. Summing up research in this period, we can show little of value. Hundreds of new plants, birds, and animals were discovered and described. Some of the plants were discovered to have medicinal value. There were no research institutes, no organized research, no universities. We may say the research contribution was the discovery that man could

## THE TREND OF RESEARCH IN AUSTRALIA WITH REFERENCE TO RESEARCH INSTITUTES

A. H. BALDWIN, *Professor of Tropical Medicine, University of Sidney,  
Sidney, Australia*

We in Australia are still in the pioneering stage. The horse and the rifle are still the equipment of the man out back. It is characteristic of pioneering times that the populace is so busy trying to live and make a permanent settlement that there is little time for scientific research. This is particularly true of medical research.

In our period of infancy from 1780-1820 when the first European settlers arrived, about 160 years ago, the whole country, not much smaller in area than the United States of America, was inhabited by some 100,000 nomads. They had no domestic animals save the dog. They had no metal tools or weapons. They possessed stone axes and spears. They neither cultivated nor stored grain. They had no

in agriculture or to modify their previous existence so as to improve their chance of survival in the modern world.

It may be wondered how this sociological factor can have any connection with tropical research. It has, however, a most important bearing on this subject. Prevalence of tropical disease is generally associated with populations, and usually large populations, living in a low economic and hygienic state. We see that in my country we had no such large population, nor did the aborigines usually dwell long enough in any one area to reap ill effects from any of their hygienic faults. They were hunters and ranged over large areas of country without permanent hut or habitation. In a very short period an area would become "hunted out" of game, and they would move on again. Consequently, and also because of their lack of contact with the outside world, the aborigines suffered from few infectious or tropical diseases. We do not know for certain the complete picture

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cholera, rabies, and typhus were apparently unknown. Tuberculosis and ordinary infectious disease, even if present, must have been rare. European settlers brought with them malaria and infectious diseases, and here as elsewhere these diseases proved fatal to the indigenous inhabitants. Fortunately neither plague, cholera, rabies nor smallpox became established. Venereal disease, tuberculosis, and leprosy have however, extracted a heavy contribution from aboriginal well being

bacilli from fleas which had fed on infected rats. C. G. Martin of the Indian Plague Commission, however, filled in the whole story. It is noteworthy that Martin was trained in Australia and did early work there on other subjects.

Transmission of dengue fever also received attention from Australian workers. T. L. Bancroft offered shrewd epidemiological evidence for the incrimination of *Aedes aegypti* as the carrier, rather than *Culex fatigans*, which had, rightly on the evidence but wrongly on its identification, been incriminated by Graham in Syria. Complete proof of the role played by *A. aegypti* was furnished by the very careful experiments of Cleland, Bridley, and MacDonald of New South Wales in 1916.

Filariasis also received considerable attention. Breinl in 1913 showed that the Queensland type had a nocturnal periodicity. Sweet, of the hookworm campaign, showed that the periodicity would be reversed by changing the sleeping habits of the patient. Mavis Walker, Heydon, and Bickhouse between 1923 and 1934 listed the potential vectors of filariasis in Australia and New Guinea. This period also saw the commencement of the foundation of research institutes. In many countries such institutes have been founded by generous gifts from families whose fortunes have been derived from trade or commerce. In Australia large fortunes are extremely rare. As a consequence, few research institutes depend solely on private bequests. Nearly all receive money from several sources, e. g., Government funds, private gifts, and university grants. In most cases, because of facilities and convenience

to or in a university or hospital the pathological or bacteriological practice such an arrangement is often unsatisfactory. One may say broadly that tropical research has been carried out in every university in the Australian Commonwealth.

			Australian
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Burnet. In

Hospital at Melbourne, in 1935, the Kanematsu Memorial Hospital of Pathology, attached to Sydney Hospital. In 1936 was formed the Sir Wm McGregor

versity, in which work

by H. D. K. Lee. In 1937 was formed the

not only exist in this country but could also increase in numbers and remain healthy

The brief period that I have just reviewed should be of interest to every one present because it is a saga of his country also. The first great and crucial experiment in medical biology is to prove that man can survive in the environment, and no further scientific work can be done until this experiment is settled satisfactorily. In the case of my land and in many others, this experiment has taken place within historical times. In more ancient countries the early pioneers figure as the heroes and heroines of mythology, may we of later times be no

garded as indicating not only a waste of medical training but actually as indicative of supreme selfishness. We can, therefore, readily understand that the type of research was of an eminently practical nature, such as an improved splint or the like. In the latter part of this 50 years, however, general practitioners blessed or cursed with the research temperament began to dabble with more scientific studies. Among these may be mentioned Joseph Bancroft. Spurred by the discovery by Wucherer of Brazil in 1806 of small worms in the urine of patients with chyluria and by the discovery of Lewis in 1872 in India, who had seen the same larval worms in the blood, Bancroft set steadily to work to try to discover the adult worm. It was not, therefore, by mere chance that in 1876 he found the adult worms in a lymphatic abscess of the arm. These were sent to Cobbold, who named them after Ban

an exciting man to live with. He bred new varieties of wheat and grapes, cultivated oysters, and indulged in many other diverse research projects.

If we sum up the research of this latter half of the nineteenth century, we would have to say it was a period of scientific exploration of the unique flora and fauna of Australia, of curiosity rather than potential

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period also occurred the world depression of unhappy memory, in which Australia was involved no less than other countries. There is no doubt that these cataclysmic events profoundly altered the course of research in Australia at the very time of its formative growth

here came experiment after experiment performed under the most rigid scientific control

The ultimate results were the proof that in New Guinea atabrine was more efficient for the prevention of malaria than quinine, the formulation of the correct dosage of atabrine for suppression, and the proof that atabrine would eradicate subtertian but merely suppress benign tertian. The early field work on paludrine was also carried out by this unit.

As a result of this work, the malaria rate fell from over 100 per 1,000 men per week to a fraction of a man per 1,000 per week, black water fever previously prevalent became almost unknown, and the death rate from malaria had to be expressed as a decimal. One can hardly decide whether to admire most the beautiful precision of these experiments by Brigadier Farley and his coworkers or the wonderful spirit and doggedness of the hundreds of voluntary human guinea pigs without whom the work would have been impossible.

New Guinea has at present no research institutes. The building of a research institute to be placed under the guidance of the School of Public Health and Tropical Medicine at Sydney has been approved. The enlargement of this latter school to nearly three times its present size has also been approved by the Commonwealth Government. It is now hoped that facilities will be available for any qualified person from abroad to study such tropical problems as may occur in our own area.

Near to Australia, we have New Zealand and Fiji, both vitally interested in tropical medicine and both carrying out work in tropical research. In Fiji is found that extremely efficient school for the training of Polynesian medical practitioners whose graduates may be found as far afield as New Guinea. It is understood also that in New Caledonia the French Government plans large extensions of research in tropical subjects.

And what of the future of tropical research in Australia? I have

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and rainfall. We must, therefore, be content to

to train many of our research workers abroad, we must encourage research workers and teachers to come to our own country, and perhaps no greater assistance could be given to us than an arrangement of some system of interchange of research workers and teachers with other countries. Such a procedure would, I feel sure, be of great advantage to my own country, but it would also give to reciprocating countries a wider view of the diverse scientific problems as modified by a different environment. Lastly, it would enable those interested in the diseases of the widely separated tropical lands to become better acquainted with one another in that fellowship of research which I believe to be an important factor in promoting the health and well being of humanity.

of Medical and Veterinary Research, at Adelaide. In 1947 were founded the Institute of Epidemiology and Preventive Medicine at Prince Henry Hospital at Sydney and the Queensland Institute of Medical Research in Brisbane.

One third of the Australian land mass lies within the tropics, although late settled, 350,000 persons of pure European descent live

tutes and other organizations during this period was the slow sorting

discovered to be widespread, and encephalitis was discovered and labeled X disease.

The Australian Hookworm Campaign was initiated, financed and encouraged by the Rockefeller Foundation, and placed under the direction of our esteemed convener, Dr W. A. Sawyer. In addition, the Campaign carried out the hookworm survey of New Guinea, and also extensive malaria and filaria surveys.

As a result of work at Australian research institutes, a somewhat geographical distribution of research could be traced. Queensland in the north studied parasitology, tropical acclimatization and the causation of the ill defined tropical fevers such as the typhus group and leptospirosis. New South Wales studied plague and dengue. Victoria concentrated on venoms and viruses.

The impact of the last World War forced Australian research workers into large scale projects with a view to the control of tropical disease, in particular, scrub typhus and malaria. This work was organized by and owes much of its success to a group of Army officers, of whom Brigadier Fairley and Colonel Keogh were the chief driving forces. Mr McCulloch did excellent work on chemicals to be used as miticides in scrub typhus and on technical methods for estimating the prevalence of mites. He formulated the necessary military drill for the prevention of the disease in field operations.

The greatest research contribution of the Army was connected with malaria. This work can be divided into entomological, therapeutic, and control developments.

On the entomological side the vectors in New Guinea were carefully studied and the most appropriate methods for their control worked out.

On the control aspect much work was done with assistance from the Council of Scientific and Industrial Research on the composition of sprays and on larvicides and finally when DDT was introduced, a very efficient method of aircraft distribution was evolved, which proved most valuable in subsequent military operations not only for the control of the mosquito but of the fly also.

The work that most contributed to military success was carried out at Cairns, North Queensland, by the Army Research Unit. From

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## INSTITUTES FOR RESEARCH AND TEACHING IN TROPICAL MEDICINE IN THE AMERICAS

MALCOLM H. SOULE, *Hygienic Laboratory, University of Michigan  
School of Medicine, Ann Arbor, Mich.*

It was only natural for those who followed Columbus to the New World to seek areas where a minimum of exertion would provide a bountiful supply of food and protection from the elements. Such areas were easily accessible in the tropical and subtropical zones. Colonies sprang up, and trade routes were charted with regular service between European ports and the frontier fully 100 years prior to a

of their insect vectors. Vast expanses of fertile land were made uninhabitable, and with the passage of time, such far reaching projects as the construction of the Panama Canal by the French were prevented by the ravages of tropical diseases.

Toward the close of the last century, considerable progress had been accomplished in an understanding of some of the phases of the etiology, epidemiology, and control of the maladies of man seething in tropical America. The causal association of *Endamoeba histolytica* with dysentery had been proven, the mosquito transmission of malaria and yellow fever had been established, and *Necator americanus* had been described. Nevertheless, infectious diseases with devastating force appeared with monotonous regularity and became the greatest single obstacle to a wholesome existence for man and the de-

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on a plan of attack. Returning students trained under the French savant and Koch in Berlin, as well as others stimulated by their publications on microbic life, urged the erection of research laboratories in the American Tropics. There were many modest beginnings before 1900. A hygienic laboratory had been organized in Rio de Janeiro in 1883. This was quickly followed by similar laboratories and Pasteur Institutes in Buenos Aires, Montevideo, Habana, São Paulo, Santiago, Mexico City, Caracas, Sucre, and elsewhere. Unfortunately, the limited funds available for the activities of these institutes were usually expended for the making of vaccines and serum rather than



for research into the fundamentals of disease. Nevertheless, there were those who envisioned the rise of institutes devoted to research and teaching rather than the commercial production of biologicals. Finally as early as 1887 had suggested the establishment of a laboratory for the study of yellow fever, and the dreams of Oswaldo Cruz and Bailey K. Ashford included schools of tropical medicine in the Tropics.

In 1909 Oswaldo Cruz literally wiped yellow fever from the confines of Rio de Janeiro. The rich inhabitants of the city, in grateful

Manguinhos in 1899 for the production of antiplague serum was designated the Instituto Oswaldo Cruz and was rededicated to the cause of tropical medicine. Dr. Cruz had gathered within the institute such outstanding scientists as Vianna, Carlos Chagas, and Adolfo Lutz. Kindred spirits attracted by the opportunity for research and the insatiable desire to aid in the eradication of the pestilences of man.

Today modest quarters and facilities are available for those members of the staff engaged in fundamental research. Intensive work in tropical diseases is given to graduate physicians in the ranks of the Federal Health Service. Special courses in tropical diseases such as leprosy, are scheduled at regular intervals with the assistance of members of the staff of the Serviço Nacional de Leprosia. These are ex-

patients eventoria

where the children of such parents are housed, provides excellent clinical material for teaching. The same is true for the instruction in trypanosomiasis. Branch laboratories or affiliates have been established such as the Centro de Estudos de Molestias de Chagas at Bambuí, Minas, and the Instituto Evandro Chagas at Belém, Pará. Dr. Emmanuel Dias pursues a very active program of research on Chagas's disease in Minas.

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paring plague vaccine and serum. Although today the study of snakes and manufacture of snake bite serum and snake venom are the principal objects of the institute, it carries on research in other fields. Foreign scientists are given facilities and are taught the methods of the institute.

Eleven medical schools in Brazil teach tropical medicine in one form or another. At São Paulo the School of Medicine provides

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are maintaining in the very best tradition the high standards he envisioned

The research activities of the Gorgas Memorial Institute of Tropical and Preventive Medicine, Inc., are centered at the Gorgas Memorial Laboratory in Panama. The functions of the institute when opened in 1929 were captioned: Research under specialists and scientists of renown, instruction at the postgraduate level of students from various countries of the world in the science of disease prevention and aid in the diffusion of knowledge by the preparation and distribution of scientific literature. Next year the Memorial Laboratory will complete two decades ably guided by the stimulating leadership of Dr. Herbert C. Clark. The activities under his direction have in every way proved to be and will continue to constitute a true memorial to the great physician, William Crawford Gorgas, whose life was replete with service to mankind.

Cuba is fortunate in having three organizations dedicated to research and instruction in tropical medicine. El Instituto de Medicina Tropical de la Universidad de la Habana was created in 1937 for teaching, research, and treatment. The courses offered by the institute include lecture and laboratory work for second year medical students and special postgraduate courses for local graduates and foreign physicians. An exchange program with Cornell University Medical College provides for the training of a selected group of students each year. Latin American students on fellowship are also regularly present. A staff of four professors with assistants and technicians is in charge of the work. The facilities of the institute, while somewhat cramped for space, include a museum with an excellent collection of specimens, maps, and illustrations as well as lantern slides and moving pictures, made on the grounds in the department of medical illustration.

retical and clinical instruction for the students in the last year of medicine, postgraduate courses in parasitology and tropical medicine

Carlos J. Finlay, is closely associated with the hospital and the

at Bogota, certain of his field work has been at El Instituto Federico Ras Acosta as its Direc

intensive instruction in tropical diseases as part of the regular curriculum in public health

The Harvard School of Tropical Medicine was opened in 1913 for the three fold object of instruction of students, research, and the organization of expeditions to investigate tropical diseases in the Tropics. Dr Richard P Strong, who came to the school as the first professor of tropical medicine following 15 years of rich experience in the Philippines, immediately proceeded to South America with three colleagues to investigate certain forms of tropical disease, particularly *Verruga peruviana*, and to collect materials for student instruction. The report of this "First Expedition to South America" was published in 1915 and is an example of the highest type of scholarly work in this field. The records of the expedition to the African Republic of Liberia and the Belgian Congo are of the same order. In 1931 an expedition went to Guatemala to investigate particularly the Central American form of the disease onchocerciasis. These and other achievements of the school under the leadership of Dr Strong have been unparalleled. On the retirement of Dr Strong in 1938, tropical medicine at Harvard was amalgamated with comparative pathology and designated the Department of Comparative Pathology and Tropical Medicine.

The creation of the School of Tropical Medicine under the auspices

time to observe the influence of tropical conditions on diseases in general. This is the first school of its kind to be established in the Americas. Today there are spacious quarters for the well equipped labora-

offered are planned primarily for graduates in medicine who wish special training in tropical medicine and hygiene. Programs leading to the degree of master of science and certificates in public health nursing and medical technology are regularly offered. Special courses have been organized on request from outside agencies, such as the Office of Inter American Affairs, which sends physicians, engineers, and technicians for instruction and field training. In addition to their teaching duties, the staff members carry on active research in their respective fields. The school stands as a monument to the ideals and aspirations of Bailey K Ashford. His colleagues and followers

believed it to be an especially appropriate location for a center of research in tropical medicine. The buildings of Instituto Nacional de Higiene Leopoldo Izquieta Perez were opened in 1941 with facilities for research on malaria, hookworm, yaws, and plague, and for the manufacture of serums and vaccines. During the war years, research routine diagnostic work and testing on the staff. It is hoped that this near future in order to keep faith with the memory of its sponsor, Dr Izquieta Perez.

There is incorporated within the structure of the Universidad Central de Venezuela the "Escuela de Patología Tropical." The staff is composed of both full time and part time members. Patients are brought from all over the country for instructional purposes. The students see a great variety of clinical material under the best of conditions. The Instituto de Protozoología, directed by Dr. Felix Pifano in the Instituto de Higiene, has a well deserved reputation in tropical diseases. During the war, under the able leadership of Dr. Arnoldo Gabaldon, El Division de Malaria at Maracay became one of the outstanding stations for teaching and research in malaria in the world. These activities are being continued, and a number of the universities in the Northern Hemisphere are sending their students to Maracay for this training.

In Argentina, at the University of Tucumán El Instituto de Medicina Regional is devoted to research in tropical medicine. The director, Dr. Romoia, in conjunction with the national department of health, presents a 4 month course in tropical diseases each year.

The diseases regularly encountered in clinics associated with medical schools mold intangibly the emphasis in the clinical years. New

1920's, a department of tropical medicine came into being with required courses in parasitology and tropical medicine for the undergraduates and elective courses at the graduate level. A certificate is awarded on the satisfactory completion of a carefully integrated program. These courses have been extremely popular, not only with

program. These courses have been extremely popular, especially in the West of the United States. During the last year, the program was expanded to include medical schools.

Through these individuals, instruction in tropical medicine particularly in parasitology, became a regular part of the teaching of all medical students, and the value to those who were later stationed in the tropics was unequivocally recognized. The Army Medical School, with Gen George R Callender at the head, participated in this program, and some of the clinical instruction was ably directed by Dr A Pena Chavarria in the San Juan de Dios Hospital, San

tor, is devoted primarily to research on leprosy. It is housed in rather sumptuous quarters with spacious laboratories, a section for the hospitalization of patients and one for the care of animals. The present staff, directed by Dr. J. Ignacio Chala H., is engaged in a

have been included.

On July 23, 1936, El Instituto Nacional de Higiene y Salud Pública de Perú was created by presidential decree. The Institute is beautifully located not far from the center of the city of Lima. Research is

institute have been maintained at a very high level by the director, Dr. Telemaco Battistini.

The members of the department of pathology of the faculty of medicine, aided by the cooperative health service, have been working on problems in tropical medicine in the oriental part of Perú and in the coastal areas. Dr. Oscar Urteaga, chief of the department of pathology, hopes to expand his division into an institute of tropical pathology in the near future.

El Instituto de Salubridad y Enfermedades Tropicales de México

THE

abundant clinical material and at Instituto el Centro Médico para el Estudio de la Onchocercosis de Huixtla, El Hospital de Arcelia and la Unidad Sanitaria de Cuernavaca. Diplomas are awarded on the successful completion of the required work, or the credit may be used for graduate degrees.

Special work in tropical medicine, both the clinical and laboratory

spirochete which is believed to be the agent of yellow fever. This organism, later shown to be identical with *Leptospira icterohemorrhagiae*, was to play an important role in the research on yellow fever for the next decade. The inhabitants of Guayaquil mindful of the part their city had played in the yellow fever and plague campaigns,

was constructed in 1939-40, has been equipped and occupied since the war

A number of losses in faculty brought about through death or retirement have occurred in recent years, both in London and in Liverpool. Able replacements have been secured, however, so that it has been possible to maintain departments in the various specialties of tropical medicine essential to a well rounded program of teaching and research. The courses of instruction in tropical medicine are in general, similar to those given before the war. Both schools offer two courses a year, each lasting 4 to 5 months and leading to the Diploma in Tropical Medicine and Hygiene. Also, students who take the full year course for the Diploma in Public Health may elect to specialize in tropical hygiene. Excellent opportunities for research are available to advanced students and fellows.

Efforts are being made both in London and in Liverpool to strengthen clinical teaching in tropical medicine. Plentiful clinical material has been available in London but has been scattered among different institutions. A special hospital where cases of tropical diseases could be concentrated is needed. In Liverpool a Tropical Disease Center for the investigation and treatment of difficult and obscure cases of tropical disease has been maintained at Smithdown Road Municipal Hospital.

#### THE NETHERLANDS

damage from the war, activities were suspended during the years of occupation and both institutions have faced shortages of personnel and equipment in resuming their former status. Teachers and research workers in the younger age group are needed and must be trained to assure the future.

Most attention since the war has been paid to immediate problems such as the instruction of young medical graduates scheduled for service in the tropics. Special courses of 3 months duration are given to meet this need. Teaching also includes the instruction of undergraduate medical students. In addition to laboratory research, the Institute in Amsterdam has played an active part in combating the postwar upsurge of malaria in North Holland and in applying new methods of control.

As part of the rebuilding program in the Netherlands a plan has been drawn up for construction of a new Seamen's Hospital in Rotterdam. Such a development will strengthen clinical instruction in tropical medicine, which students at the Institute in Leiden must obtain in Rotterdam.

#### BELGIUM

The Prince Leopold Institute of Tropical Medicine established in Antwerp in 1933, is the chief Belgian center for training and research.

Jose, Costa Rica The hospitals of the United Fruit Co cooperated in full measure

There are many medical schools in the United States and Canada, as in out is 1

institutions entitled to special consideration McGill University provides excellent training leading to a diploma of tropical medicine on the completion of two academic terms in residence at Montreal and a year of practical clinical experience in the Tropics Columbia University could not avoid an active interest in tropical diseases The visitors passing through the port of New York brought at one time or another examples of all the exotic diseases, thus providing clinical material which stimulated student and faculty interest Johns Hopkins has long attracted students from foreign lands because of the outstanding research in parasitology and tropical medicine The medical students at the University of Cincinnati have been unusually fortunate in having as a teacher Dr T LeBlanc, who not only presented the problems of tropical disease in the classroom but also organized trips to Puerto Rico where the students could see for themselves The University of California and the University of Southern California have made fundamental contributions in this field In the late 1890s Dr Novy at the University of Michigan became enamored with the agents of tropical disease The publications of his group on trypanosomes and spirochetes extending over a period of 50 years have been noteworthy

The training of personnel for service in teaching and research and active participation in research has now become a function of various branches of the armed services and other Government agencies in the United States The Army Medical Department Research and Graduate School the Naval Medical Research Institute and the National Institute of Health all have unusually spacious quarters and equipment for this purpose

The guiding influence of the Pan American Sanitary Bureau in encouraging and strengthening programs in tropical medicine in the Americas has been most commendable and should be gratefully acknowledged The Institute of Inter American Affairs has used the facilities of many of the institutions described in its most laudable training program

In closing due credit must be given to the Rockefeller Foundation and also the Rockefeller Institute The foundation has organized and unstintingly contributed to such programs as the eradication of *uncinariasis malaria* and yellow fever it has sponsored the training of personnel it has aided in the construction and staffing of medical schools and schools of nursing in the tropics and in popularizing information concerning tropical diseases it has been of immeasurable service to mankind



special courses in tropical medicine, and maintenance of facilities for clinical investigations

The principal course offered extends over a period of 4 months and leads to a Diploma of Tropical Medicine. It has been attended by an average of 20 students. Another course with lectures presented in a popular manner is offered to lay individuals who expect to work in the tropics. This course lasts 8 weeks and has had an average attendance of 45 to 50 students. The courses in tropical medicine in these territories of tropical problems constitute a welcome and significant contribution in this field.

### SPAIN AND PORTUGAL

Except for the general curtailment in exchange of scientific information and materials, activities in these countries were not greatly affected by the war. The National School of Health in Madrid is the principal center in Spain for teaching tropical medicine. Although research in tropical medicine, malaria, and part of the three research in tropical Colonial Medicine

is awarded. The course is compulsory for doctors planning to work in the tropical colonies. Attendance averages about 60. Clinical instruction is given at the Colonial Hospital, where interesting cases are brought in from the tropics for study.

The Institute in Lisbon has departments in the various tropical specialties which carry on research programs. Plans have been drawn up for a new building and it is expected that construction will be finished within the next 3 years.

### ITALY

The principal special facilities for training and research in tropical medicine in Italy are furnished by the Superior Institute of Public Health in Rome. The fine modern building erected in 1934 suffered some damage from air attack during the war but is essentially intact. In the past year an extra floor has been constructed to provide addi-

## 1 RESEARCH AND TEACHING INSTITUTES

in tropical diseases. The Institute did not suffer any phys during the war, and its operations were not interrupted. A graduate course in tropical medicine lasting 4 months is o a year. From 25 to 50 students attend each course, and r a Diploma of Colonial Medicine. This instruction is con physicians planning to practice in Belgian Congo.

Another more general course, also lasting 4 months and c a year, is given for nurses, sanitarians, missionaries, an individuals going to the tropics. About 100 students r courses, which include elementary laboratory work as well. Instruction is given both in French and in Flemish.

A clinic with 50 beds for patients with tropical diseases i

### FRANCE

The principal source of French research in the field medicine has always been the system of Pasteur Institutes.

medicine, they have never played the role of teaching

medicine is a handicap in bringing the subject to the fore in France.

In common with all educational and research institutions those where tropical medicine has been prominent have times in the postwar period. For example, funds for general expenses such as animal colonies have been limited. It has been largely impossible to obtain foreign exchange supplies and equipment which must be purchased abroad. Of the inflation and the lag in salaries it has been diffi

uation improve. In the meantime, teaching and training medicine suffer until new blood and new life can be infu

### SWITZERLAND

cal Parasitology and Helminthology of the Academy of Medicine. It serves to train specialists for malaria control units and to conduct research in all phases of medical parasitology.

### GERMANY

Before the war the Institute of Seamen's and Tropical Hygiene in Hamburg was an outstanding center for training and research in tropical medicine. In 1943 and 1944 the Institute was severely damaged from air attacks and had to evacuate its activities to other locations.

Although reconstruction has not been possible since the war, partial work repairs have been made on the old central building and the Institute has reoccupied its former site. In spite of hardships and handicaps, work is being carried out in all departments. Instruction was resumed in the fall of 1946 with the scheduling of classes for graduate German physicians.

Most of the library and museum has been salvaged. Even the strains of schistosomes, long maintained with their snail hosts, have been

of German economy and education as a whole.

### SUMMARY

Although necessarily brief, this review is sufficient to indicate the facilities for research and training in tropical medicine in Europe: many and varied, and that instruction is available in a number of different languages. As a rule, the programs of the institutions concerned with this field are designed to serve the national needs of the country in which the institutions are located. However, all may have an international aspect as well, not only because of the worldwide character of tropical diseases but also because many of the students come from foreign lands.

### ABSTRACT OF DISCUSSION

**Dr J. RODHAIN (Belgium).** Dr McCoy said that we suffered real damage in Antwerp, but we had some. We suffered much difficulty because we had to kill our animals just as the war came over Belgium, and during the hard times after the occupation of Belgium we had many difficulties. Still we will do our best to maintain good name for our institution, and now we are started again with good man.

I'd like to add another few words about training for the medical men. After the medical men have finished the courses in Antwerp and gone to the Congo, they must make a change from the laboratories to the big hospitals for the natives and this completes the course.

Courses for medical students are given but no formal graduate instruction is offered at present. However, considerable opportunity is afforded for special students to undertake investigative work in subjects related to tropical medicine. Since the war the Institute has been particularly active in malaria control. Staff members are participating in the campaign to eliminate the disease from Italy and are conducting laboratory and field research with DDT and other new insecticides.

Another institution in Rome concerned with tropical medicine is the Institute of Malariology. It functions mainly to give instruction to physicians in the medical aspects of malaria.

### GREECE

Because of the disturbed political and economic situation, the National Institute of Hygiene in Athens has had considerable difficulty

which has so markedly reduced the disease in Greece during the past few years. Since the war many of the staff members have had opportunity to travel and study abroad. The nucleus for a successful institution is present, but national stability and more powerful support are required for it to prosper.

### OTHER BALKAN COUNTRIES AND RUSSIA

Very little information is available to the writer concerning recent developments in tropical medicine in other Balkan countries, or in the Soviet Union. The Institute of Hygiene in Zagreb, Yugoslavia, began to function again immediately after the war. Its teaching program includes training in various branches of tropical medicine,

to have a very active teaching program as well. Do not - - -

# PHYSIOLOGICAL ADAPTATIONS OF DWELLERS IN THE TROPICS

G MONGE, L CONTRERAS, T VELASQUEZ, C REYNAPARJE, C MONGE, Jr  
and R CHAVEZ, *Institute of Andean Biology, University of San  
Marcos, Lima, Peru*

The relationship between man and environment must be the subject of careful scientific studies. Castellani (1), Dill (2), Adolph et al (3), Mills (4), and Lee (5) have contributed to the knowledge of the physiology and acclimatization in hot climates, as much as the important researches carried out during the last war.

We have endeavored to approach the problem of physiological adaptations in the tropics by studying men going down from the cold high tropical plateaus of the Andes either to the warm zones of sea level (coast) or to altitudes approaching sea level (jungles). Before going any further, it is important to emphasize an accurate point of view. The word "tropical" is usually taken in the sense of "hot climate" exclusively. We feel that this is not exact and that it should be corrected.

The notion of "climatic aggression" rests at the problem's foundation. The man who moves to the tropical lowland is

tization" have thus an actual meaning. A precise terminology becomes necessary to avoid confusion.

Millions of men in America, Asia, and Africa, live in tropical zones or near them, but at altitudes sometimes as high as 17,000 feet. It

can indigenous societies, and then by progressive descent, they arrive at the temperate or warm climates of sea level. Tropical sun is a constant for all those climates, but in the high plateaus it is cold during the night and in the shade during the day, while the density of the atmosphere is almost half the density of polar or equatorial atmosphere, and oxygen tension may be only half the pressure at sea level.

Those high altitude tropical climates are not really well described in the current textbooks of climatology, which only study the climate of moderate altitudes. In chart 1, taken from J Broggi (7), we show the main characteristics of the three climatic zones in which we have studied the man of the Tropical Andes: Morococha, Huancayo, and Lima. Our next investigation will be carried out in Iquitos (jungle).

## SECTION II

# Tropical Climatology and Physiology

### Session 1 TROPICAL CLIMATOLOGY AND PHYSIOLOGY

*Tuesday, May 11--8 15 to 10 25 p m*

*Departmental Auditorium, Room B*

The meeting was called to order by Dr David B Dill, convener of section II, with a word of welcome to those present and a brief review of the pertinent parts of the rules of procedure The meeting then elected an honorary chairman, a chairman, and two vice chairmen The completed list of officers was as follows

OFFICERS

..

Colonel Wesley Cox, United States, vice chairman

Dr David B Dill, United States, secretary

Dr Milton O Lee, United States, assistant secretary

Dr Shattuck presided while the following papers were presented

the high plateaus of the tropical Andes show such deviations from the normal sea level standards, that it can be stated that Andean man belongs to a climato physiological variety of the human race (Monge).

Actually, fitness at high altitudes involves shift from the normal sea level values to new physiological patterns. Therefore, in order to avoid confusions we have to consider several "normal" values for different altitudes. They are shown in our charts. Those interested

blood volume is found to be increased (35 percent) on account mainly of an increased quantity of red blood cells. The arterial saturation is low as well as the contents of free and combined carbon dioxide, the alveolar oxygen tension is higher than before acclimatization, bilirubin and pyruvic acid values are increased (8), the circulatory response

We will proceed now with our findings during the changes of environments

tado, Merino, and Delgado (6). It is interesting to observe the rise of the red blood cell count, hemoglobin, hematocrit, reticulocytes, bilirubin, and blood volume when the subjects were taken from Huanayo to Morococha. Only the plasma volume decreased slightly. The

interpreted as though the adaptive process has not yet been achieved.

With the subjects brought down to Lima in 5 hours, a progressive diminution of the hematologic factors was observed during 8 weeks of observation. The red blood cell count, as well as the hemoglobin and hematocrit values, fell well below the normal values for sea level keeping a linear relation with the time elapsed. This fact had been

to attain the normal sea level value. The total blood volume decreased progressively, becoming normal at the end of 8 weeks, the red cell

destroyed in order to reach the sea level equilibrium. The values of

PLACE	Altitude feet	Longitude	Latitude	PRESSURE		TEMPERATURE				Barometer readings	Climate	
				Barometer	O <sub>2</sub>	Max	Min	True Avg	Obs. L. 24h			
LIMA	510	77° 02' W	12° 03' S	750	156	375	76	84.0	22.7	3737	6640	Dry Coast
IQUITOS	347	75° 12' W	5° 45' S	752	157	370	176	51.80	13.24	2673.5	6010	Moist Lowland

Chart I

While in the tropical altitude climates the average temperature range is  $40.9^{\circ}\text{C}$  (extremes  $+30.9^{\circ}$  to  $-10^{\circ}\text{C}$ ), on the coast it is  $22^{\circ}$  (extremes  $+32.5^{\circ}$  to  $9.6^{\circ}\text{C}$ ), and in the jungle Iquitos it is only  $13^{\circ}$  (extremes  $+37^{\circ}$  to  $17^{\circ}\text{C}$ ).

In order to study the effect of tropical climate of the lowlands we have selected clinically and radiologically in Huancayo (10,170 feet, 3.3 km, yearly mean temperature,  $12.05^{\circ}\text{C}$ ) a group of 10 soldiers, born in the high plateaus and with ages ranging from 19 to 23 years. They were taken to Morococha (14,900 feet, 4.9 km, yearly mean temperature,  $6.25^{\circ}\text{C}$ ) where they sojourned for 15 days, after which they were brought down to Lima (sea level, yearly mean temperature,  $18^{\circ}\text{C}$ ). The research began during the summer in Huancayo, was continued for 15 days in Lima.

Next 4 months in Lima, jungle Iquitos (317 feet, comparative physiology of both climates will allow us to distinguish the temperature factor from the altitude factor, which will remain fairly constant.

dis  
vol  
acid  
(Edwards), pyruvic acid (fatigue laboratory manual), pH, blood  
equilibrated at  $p\text{CO}_2/40\text{ mm}$   
metabolism (open circuit),  
polar leads, radiology of  
method.)

Statistical studies of the values found will be presented in the individual papers soon to be published. For the purposes of brevity, we have considered here only the mean values.

The physiological and biochemical characteristics of men living in



tained in Huancayo, but it falls down in Lima, right after the descent. The behavior of the pyruvic acid is noteworthy. The pyruvic

ues. It remains high throughout 8 weeks, becoming normal at the end of the fourth month.

*Acid base balance*—We have studied the acid base balance only in venous blood (chart 4) and observe that the free  $\text{CO}_2$  values are the same in Huancayo and Morococha, but they increase in Lima remaining high throughout the experiment. The bicarbonate is lower in Morococha than in Huancayo and keeps constantly low throughout the stay in Lima. On account of these variations, the pH shows a shift to the acidity zone during all the adaptive period. We can see from the relations of these physicochemical changes that in Morococha acidosis tends towards an intermediate zone between the fixed and respiratory types while in Lima it tends towards the respiratory type (graph 2).

*Basal metabolism*.—The study of basal metabolism (chart 5) provides normal values both in Huancayo and Morococha as well as in Lima. The respiratory quotient tends to go up in the later determinations in Lima. The pulmonary ventilation is found to be increased in the altitude, diminishes at sea level, but it increases again later. The pulmonary ventilation did not reach in Morococha the figure of 8.3 liters per minute found as normal in some cases by T. Velasquez (9) in natives.

*Electrocardiography*.—The normal electrocardiogram of the altitude dweller was described by Saenz (10), Rotta (11), and Alzamora and Monge in Huancayo (10,170 feet) (to be published).

As a general rule in the altitude normal electrocardiograms, the AQRS in most of the subjects shows a deviation toward the right ( $+90^\circ$  and  $+180^\circ$ ). In our subjects in Huancayo, a vertical position was frequently found.

In our soldiers the electrocardiograms taken in Morococha showed such alterations as a measurable elevation of the ST segment with inverted or diphasic T wave in the unipolar precordial leads, mainly V1, V2, and V3, but sometimes even in V5 and V6. In general, in most of the subjects a right axis deviation was present, sometimes beyond the normal. All these findings are in relation with the adaptive phase to the altitude.

The ECG's taken in Lima immediately after the descent and then every 15 and 30 days during 4 months, showed definite alterations. A major modification from the altitude ECG's was seen in the ECG's taken in Lima right after the descent (3 days). The T waves, altered in Morococha, became normal and there was a tendency of the ECG's to attain a normal shape. In further examinations, a constant enlargement of the QRS amplitude and especially of the T wave voltage was found, sometimes in all leads. In some cases, the

increased somewhat, but it dropped during 8 weeks of observation without quite reaching the normal standard for sea level. The indirect bilirubin shows parallel variations.

**Biochemistry**—Chart 3 shows that the low glucose contents found in Huancayo are somewhat higher in Morococha. After the descent to Lima, blood glucose values go up progressively in successive determinations but without reaching the sea level normal figures. The lactic acid goes up in Morococha, as compared with the values of

PHYSIOLOGY	Morococha men Altitude 14900 ft	Quito men Altitude (2230) ft	Huancayo men Altitude 10170 ft	Morococha men from Huancayo	Lima men from Huancayo 1st W	Lima men from Huancayo 3rd W	Lima men from Huancayo 8th W	Lima men from sea level
Red Blood Cells ml per cu mm	4.45	5.47	5.45	4.05	5.57	5.37	4.70	5.14
Hemoglobin g per 100 ml	20.36	18.82	18.85	17.95	18.49	15.95	14.30	16.00
Hematocrit and critical point	59.90	54.70	50.36	54.43	50.67	49.46	43.30	48.80
Erythrocytes per mm <sup>3</sup>	1.5	0.8	0.47	1.94	0.77	0.25	0.4	0.5
Total Bilirubin mg per 100 ml	1.56	1.42	0.84	0.89	0.9	—	0.83	0.72
Direct Bilirubin mg per 100 ml	0.46	—	0.16	0.38	0.26	—	0.15	0.37
Indirect Bilirubin mg per 100 ml	1.10	—	0.68	0.46	0.63	—	0.50	0.35
Blood Volume L per 70	4.98	4.45	5.36	5.58	5.55	5.49	5.17	5.21
Plasma Volume L per 70	2.85	2.78	2.55	2.29	2.64	2.67	2.80	2.82
Red Cell Volume L per 70	4.29	3.36	2.79	3.25	2.87	2.79	2.35	2.34
Blood Volume ml per kg	120.8	108.7	87.2	90.64	89.67	88.11	81.70	86.5
Plasma Volume ml per kg	46.1	45.9	41.46	38.77	42.83	42.67	44.28	47.1
Red Cell Volume ml per kg	74.1	54.7	45.45	53.78	46.45	44.75	37.40	38.8
Total Hemoglobin g per 100	14.6400	13.000	10.501	10.282	11.452	10.726	11.799	12.600
Total Hemoglobin g per L	252	207	1473	1676	1480	1291	1144	112

Chart 2

BIOCHEMISTRY	Morococha men Altitude 14900 ft	Quito men Altitude (2230) ft	Huancayo men Altitude 10170 ft	Morococha men from Huancayo	Lima men from Huancayo 1st W	Lima men from Huancayo 3rd W	Lima men from Huancayo 8th W	Lima men from sea level
Glucose mg per 100 ml	57.0	75.0	64.0	71.0	80.0	85.0	85.0	105.0
Lactic Acid mg per 100 ml	14.07	12.59	12.78	14.30	11.5	12.78	10.35	11.0
Urea Acid mg per 100 ml	2.13	2.16	1.52	1.44	—	2.24	2.20	1.37

Chart 3

largest amplitude of the complexes was observed mainly in the un-

intermittent oscillations. After 4 months some of the E C G's became normal. After 6 months one of them has shown a return to the Morococha aspect.

During the stay in Lima, a gradual left axis deviation was present in most of the subjects, but again, the variation was oscillatory. The electric position did not always follow closely the axis variations although there was a tendency toward an horizontal position all along the adaptative process. In one case there was a definite return to the initial vertical position.

*Radiology*—We have to point out, as has been stated by Kerwin (12), Miranda and Rotta (13) in Morococha that there was an enlargement of all the heart diameters as compared with those of sea level. In Huancayo the increase was much less marked. A frequent radiological pattern showed a 19.5 percent increase of the transverse diameter in 60 percent of the cases in Morococha (Rotta). In our cases, some enlargement was observed in Morococha, and then, during the stay in Lima, the diameters tended to increase gradually in some subjects even after 4 months. We observed an increase up to 40 percent in the vascular pedicle, 19 percent in transverse diameter and 36 percent in cardiac area. In two cases the diameters diminished.

was observed from the beginning, so that a definite rule cannot be established.

*Clinical data*—We present the main clinical details in chart 6. The arterial tension and the bradycardia of our subjects, a fact already

### DISCUSSION

In the study of the adaptation of Andean man to sea level environment, important physiological deviations are found.

To become a sea level man, an altitude dweller has to change his hematic equilibria to patterns different from those which constitute the normality in the cold altitude. Thus, 2 liters of blood are necessarily destroyed in men from 14,900 feet altitude.

In our observations we found that 214 grams of hemoglobin were destroyed.



There is one point that I think should be investigated if Dr Monge has the opportunity and that is the possible sludging of the blood when these men go f

this marked decrease  
dently some interfere

go down and it is quite possible that the so called sludging, as described in the magazine, Science, last fall, occurs. That, of course, would block capillaries and produce serious impairment of the individual circulatory system. In addition to that, there is a second

I  
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infections at sea level than at higher elevations? (Dr Monge replied in the affirmative)

The respiratory infections are dependent entirely on the phagocytic functions as the first line of defense, and almost the only line of defense, against the initiation of infection. Therefore, this stage in which we have almost complete quiescence of bone marrow activity the stage from the first week to perhaps a whole month down at sea level, would be one of very, very sluggish bone marrow both in the production of red cells and hemoglobin and in the production of the phagocytes. So there is a possibility that Dr Monge will find, in this matter of vertical adaptation of man, that when he goes down he faces very severe hazards other than just temporary interference with his physical activities.

The alteration in heat production, as shown by the basal metabolic rate, is to be expected. These individuals going down to sea level

also the ability to take the heat from the internal regions of the trunk out to the extremities for dissipation. Therefore the impairment of the efficiency of the circulation of the blood through the

particularly when they do it in less than a few hours by air, they face real difficulties.

Dr M. NARASIMHA RAO (India) I congratulate Dr Carlos Monge on the excellent piece of work he is doing in Peru. His work is all the more important in that it is related to some fundamental studies like the ones on hemopoiesis and altitude.

Some time ago we, in South India, noted that the size of the red blood cell does not vary significantly with altitude. The red cell

	Morococha	Quaya	Huancayo	descente	Lama	Lama	Lama	Lama
Pulsations per minute	64	61.2	63	64	47	51	57	65.88
Respirations per minute	—	—	16	16	15	15	16	—
Body Weight Kgrs	—	—	61.3	61.7	61.5	63.0	63.4	—

Chart 6

cases of chronic mountain sickness showed the same fact. Furthermore, the blood sugar contents, low in the altitude, rising in Lama,

8 weeks were still in the adaptative period, in other words, acclimatization had not yet been attained.

The same can be said, as far as the modifications of the physiology of the heart are concerned, such as the bradycardia found after the descent to Lama and the E C G abnormalities, which suggest so great deviations of the electric potentials that in some cases they might be taken as pathological but the regressive tendency of them to nor-

Graybiel et al (20) On the other hand, we have to emphasize the observed change in the position of the heart which from a vertical

conditions

The general subjective condition of our men did not change with their removal from Huancayo to Morococha and Lama. In Lama no slackening of their capacity for work was observed. They were performing a moderate work and all of them behaved as perfectly normal beings. There was no question of lack of food or vitamins. They

# TROPICAL DETERIORATION AND NUTRITION A DISCUSSION BASED ON OBSERVATIONS ON TROOPS<sup>1</sup>

ROBERT E JOHNSON, M D, D PHIL (Oxon), *The U S Army Medical Nutrition Laboratory, Chicago, Illinois, (an installation under the jurisdiction of The Surgeon General, Department of the Army)*

The substance of this paper is taken for the most part from a paper published in 1947 (Kark, Aiton, and Pease, Bean, Henderson, Johnson, and Richardson, 1947), which discussed medical surveys of American troops in the Pacific and Indian troops in Burma and India, with emphasis on tropical deterioration and nutrition. The syndrome variously termed "tropical deterioration" and "tropical neurasthenia" is said to be characterized by "anorexia, easy tiring, palpitation, languor, variable abdominal pains, often unexplained nausea, vomiting, and diarrhea. Various functional symptoms are present. The systolic blood pressure is below normal. The basal metabolism is little, if any depressed. The organic structure shows no constant abnormality" (Reed, 1942, Price, 1940).

Deterioration in the Tropics has been ascribed to heat and humidity diseases, alcoholic intemperance, improper food, lack of physical exercise, and psychological disturbances. It is not known whether physical, physiological, and psychological types of deterioration are separate entities or whether such forms as do occur in the Tropics are fundamentally different from those in temperate zones. If true differences exist are they qualitative or merely quantitative? Are tropical influences special or do they merely accelerate a nonspecific process? These points as they affect civilians have been discussed by Shattuck (1938).

The disastrous failure of our arms in the jungles of Malaya, Burma,

1. The opinions expressed in this paper do not necessarily represent the official views of any governmental agency

was harmful, and those white adults who were unfortunate enough to have to earn a living in the Tropics had to return to temperate zones every 2 or 3 years to recuperate (Castellani, 1938).

<sup>1</sup> The opinions expressed in this paper do not necessarily represent the official views of any governmental agency

measured 7.08 microns at sea level and 7.14 microns in those living at 6,000 feet above sea level. It would be an interesting observation if Dr. Monge could include the measurement of the red cell size also in his numerous tests on the same subjects moving from one altitude to another. (Reference: Rao and Rao, 1942 Indian J. Medical Research, vol. 30, p. 65.)



Some of the stigmata specially searched for are listed in table 1

TABLE 1—*Biochemical status of a motor transport unit a mule transport unit Japanese prisoners and a Gurkha regiment*

[Figures represent average values]

Substance measured	Motor transport	Mule transport	Japanese prisoners	7/10 Gurkha R. fls
	14.9	13.1	12.4	14.4
	6.1	5.7	5.6	5.3
	107.6	99.0	98.0	104.0
	5	2	7	7
	2	1	1	2
	2	2	6	8
	34.0	14.0	4.0	6.0
	8.0	8.0	9.0	15.0
	5	4	7	6

*Test of physical fitness*—The Canadian team employed the "pack test" of stamina for hard physical work (Darling et al, 1944) and the United States team used a "step test" which is the same in theory but in which the subject does not carry a pack. Both variants of the test yield scores which increase with improvement in physical training for hard work and decrease with deterioration in physical condition as in acute caloric deficiency (Kark et al, 1944).

*Issue of rations*—Subsistence policies and problems were discussed with quartermaster officers, and inspections were made of subsistence items to determine particularly the use, keeping quality, and stocks of important items. Observations on food preparation, acceptability preferences, and palatability were made.

*Dietary history and nutrient intake*—The four types of information obtained in dietary interviews with each test subject included (1) A  
at all,

and (4) items of food eaten in addition to those issued by the army. From these together with data on ration issues the intake of nutrients was calculated (Berryman et al, 1943, 1944).

*Biochemistry*—The teams traveled with portable laboratories similar in construction and identical in methods (Johnson, 1945). For the present surveys most of the reagents were prepared in large batches by one laboratory in North America and were divided between two teams.

Specimens of blood and urine were obtained before breakfast according to the following schedule: 5:40 a. m., reveille, 5:50 a. m., subjects report without eating and before emptying bladder, subjects empty bladder into latrine and drink approximately 1 pint of water,

The experiences of the latter half of the war, however, disproved many of the beliefs and customs of white settlers in the Tropics (Fairley, 1945). With the development of effective methods for con-

out of hot, humid climates for as much as 5 years, thousands of white soldiers working in the midday sun without solar topees, many of them bareheaded, and finally in the Philippines white American troops who were fit and well after months of continuous fighting and physical labor in the jungle.

The observations reported here on the health, fitness, and nutritional state of troops in Burma and the Pacific are among the few that have been made in the Tropics by modern quantitative methods on physically active young men in whom disease had been well controlled and whose supply of food was ample. The only similar studies of which we are aware are those of Lee (personal communication, 1945) on Australian troops in New Guinea.

### METHODS

Our work was done during early 1945 by two separate teams, who had trained together and who used almost identical methods. One team was loaned by the director general of Medical Services, Canadian Army, to allied land forces, southeast Asia, and operated in India and Burma to study Indian soldiers exclusively. The other was sent out by the offices of The Surgeon General and Quartermaster General,

tional pertinent information was obtained by examination of hospitalized patients and by interviews with local personnel other than those subjected to the full battery of tests. Observations were also made on environmental conditions under which the men lived and on their daily work and activities. A general description will be given of methods employed by both teams.

*Medical history*—A history obtained from each man stressed diseases and conditions which might predispose to or precipitate nutri-

6 a m to 7 30 a m, specimens of venous blood are drawn, 7 30 a m, subjects empty bladders into cups. Time and volume of urine are noted and samples of urine are stabilized with oxalic acid. Analyses of blood included hemoglobin, serum protein, serum ascorbic acid, and serum chloride. Analyses of urine included chloride, ascorbic acid, and creatinine.

whole organizations or garrisons

*Comparative data from field trials*—In 1944 there were in North America and the Pacific 100,000 troops in the field.

troops. The adequacy of various combat rations eaten by native working infantry troops was determined by interviews, observations of tactical efficiency, clinical examinations, tests of physical fitness, bio-

field trials to include men under stress of environment, combat, and

survey  
present

groups had participated in at least one of the 1944 trials, that the biochemists of both teams had worked together in both trials, that the criteria used in the clinical examinations were the same, and that all clinical examinations in one theater of operations were made by a single medical officer.

#### GENERALIZATIONS BASED ON COMPARISON OF TROOPS IN NORTH AMERICA, THE PACIFIC, AND BURMA

##### EFFECTS OF STRESS, AND TROPICAL DETERIORATION

The effects of the stress of combat on well fed troops among whom tropical diseases were well controlled may be seen by comparing United States troops in rear areas of the Pacific with those recently in combat. Iwo Jima had been taken 12 weeks before the survey team arrived, and battles on Okinawa and Luzon were still in progress. There had never been any fighting on the island of Hawaii, and at the time of survey Guadalcanal had been secure for 2 years and Guam for 6 months.

Symptoms referable to the skin, gastrointestinal tract, and neuromuscular systems tended to be commoner in the groups under stress.

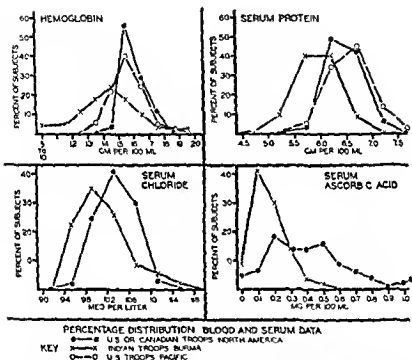


Figure 1.—Percentage distribution curves for data on hemoglobin, serum protein, serum chloride, and serum ascorbic acid for United States troops in the Pacific (300 men), Indian troops in Burma (1,000 men), and troops in North America. For the latter, hemoglobin values are for Canadian infantry (150 observations); serum chloride values are for United States infantry battalion in Colorado (600 men); and serum ascorbic acid values are for United States infantry in the Arizona desert (149 men).

of combat. Positive physical findings were also commoner, especially conjunctivitis, gingivitis, poor oral hygiene, folliculitis, malaria, fungus infections, and epidermophytosis. Weight was lowest on Iwo Jima and Luzon. On the other hand, physical fitness was actually better in those exposed to stress, and biochemical status was strikingly different in the two groups.

Although there is no quantitative way of measuring morale one can usually determine from conversations with commissioned and noncommissioned officers and by personal observation of the troops whether it is good or bad. In the Pacific, morale was good where men had a job to do of obvious immediate importance, and in general was better the further forward they were. On Hawaii and Guadalcanal, morale was poor, on Guam, fair, and on Iwo Jima in the 3d Infantry Division on Luzon after 14 consecutive weeks in the front line, it was excellent.

These United States troops recently in combat were adequately fit men without serious disease, who showed some wear and tear but could still carry on well and do their job efficiently. A different picture was seen in the men of one Indian unit, who deteriorated badly as a result of prolonged and severe stress accentuated by an inadequate diet.

Two Indian units attached to the same infantry brigade worked in the same area and served the same rations. Marked differences were demonstrated,

although both units had been on active service in Burma for similar periods of time, both had received the same rations, and at least from the Army's point of view both had been engaged in the same work in the same country under exactly similar climatic conditions. One unit used motor vehicles to transport material and other packed supplies on mules and had to march to their objective.

Analysis of the stresses which affected both units showed that the muleteers (41st Animal, Mule Transport Company, Royal Indian Army Service Corps) expended over 4 500 calories daily in marching, in taking care of the mules and in constructing defenses. They had little rest, since sleep in the field was often disturbed by the enemy. During the day they were exposed to sun, wind, and rain. They subsisted on a standard Army ration which was often reduced by  $\frac{1}{3}$  or  $\frac{1}{2}$  because of the tactical situation. They did not supplement their rations to any extent and lack of opportunity to use mosquito nets and quinine requirements they were not supplied. They used sterilized water when watering their mules. Malaria was common because of contact with their animals, thorn scratches, insect bites, and lack of opportunity to bathe. Malaria and diarrhea rates were high.

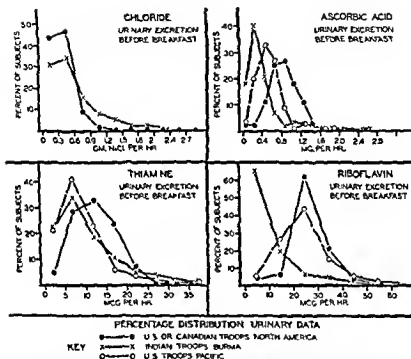


Figure 2.—Percentage distribution curves for data on chloride, ascorbic acid, thiamine, and riboflavin excretion in samples of urine collected before breakfast from United States troops in the Pacific (300 men), Indian troops in Burma (1,000 men), and United States infantry in Colorado (600 men)



## CORRELATION BETWEEN THE EXCRETION BEFORE BREAKFAST OF VITAMINS IN URINE AND CALCULATED DAILY INTAKE

## KEY

- U S UNITS NORTH AMERICA
- U S UNITS PACIFIC
- x INDIAN UNITS BURMA

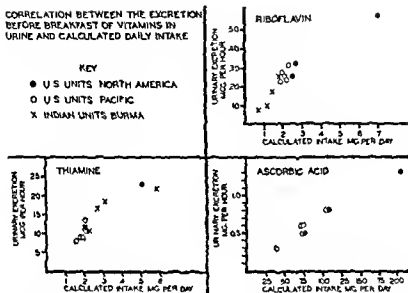


Figure 3.—Correlation between calculated average daily intake and measured excretion of riboflavin, thiamine, and ascorbic acid in urine before breakfast. States troops in Pacific and North America represent averages for groups of approximately 50 men each. Points for Indian troops represent averages for groups of 39, 55, 72, and 140 men. Troops excreting large amounts of riboflavin (50 micrograms/hour), thiamine (93 micrograms/hour), and ascorbic acid (1.4 milligrams/hour) were receiving vitamin pills along with their regular rations.



cal analyses are usually carried out on blood and urine. Fitness, efficiency, and work output are rarely estimated in any quantitative manner. In the past, implicit or explicit assumptions have frequently been made (1) That there are close correlations among the different types of data collected by the methods outlined, and (2) that it is possible to set minimal values below which there is ill health (Adamson et al 1945). Our present observations allow several conclusions to be drawn on these points:

The first general conclusion is that within a "normal population," i.e., one that is able to work efficiently and is not ill enough to be hospitalized, there is reasonable correlation between the average intake of various substances for a period of months and their average con-

and would be anticipated on *a priori* ground (Johnson, Henderson, Robinson, and Consolazio 1945). It should be emphasized that this correlation was found for large groups, and that the range

"normal population" as defined in the preceding paragraph, the measurements made in the present surveys showed few correlations among biochemical values, physical fitness, and clinical findings.

The relationships between individual clinical stigmata and biochemical status and between clinical stigmata and step test scores were investigated in United States troops. In the first place, the correlation between step test scores and biochemical values were determined.

The magnitudes of the correlations were then averaged and compared with the "normal" and "abnormal" ranges. The magnitudes of the correlations were then compared by comparing them with pooled intra-island variances. Only a few statistically significant correlations were found (table 2). Slightly low step test scores correlated with gross conjunctivitis, gingivitis and poor oral hygiene. Slightly low riboflavin values were correlated with poor oral hygiene and a statistically significant correlation existed between step test scores and oral hygiene.

The relationship between individual clinical stigmata and biochemical values in Indian troops is shown in table 3. In the United States troops in the Pacific, statistically significant correlations were found between (1) increased excretion of uric acid and (2) decreased excretion of uric acid.

from his studies on civilians and troops in Australia and New Guinea that tropical deterioration is not a specific entity to be differentiated from deterioration seen elsewhere. With his conclusions, we are in general agreement and can contribute to the question in two ways.

First, white troops were fighting winning battles after continuous presence for as much as 3 years in severe tropical environments. They received good medical care, led an active life, and maintained satisfactory health. Nevertheless, psychological changes for the worse were present in some rear areas where men were isolated for no reason satisfactory to them, and yet these men were facing less danger, discomfort, and disease than those farther forward, in whom morale and performance were good in spite of severe climatic stresses.

Second, under certain well recognized types of stress, men will react and deteriorate similarly in tropical and in temperate or cold environments. The survivors of the United States 38th Infantry Division on Luzon showed weight loss and other effects of a long campaign. Nevertheless, they had good efficiency, high morale, and a generally good nutritional state. In the mule transport company in Burma, we had a group showing deterioration along with diseases

to stresses beyond their breaking point (Kark, Johnson, and Lewis, 1945).

We conclude that the factors leading to deterioration in military personnel in the Tropics are in some cases the same as they are in temperate or cold environments. We have seen little evidence of a specific effect of the Tropics except for skin diseases, especially malaria and fungus infections. Neither have we seen cases of deterioration which could be diagnosed as purely tropical or climatic in origin. When deterioration does occur, as in the mule company, it is our impression that it may be more disabling than elsewhere because of the natural environmental handicaps which exist in warm humid climates.

#### INTERRELATIONS AMONG DIETARY INTAKE, BIOCHEMICAL MEASUREMENTS, PHYSICAL EFFICIENCY, AND CLINICAL FINDINGS

In most nutrition surveys of general populations, information is collected in several ways. Dietary intake is estimated by laboratory analysis of meals, by calculation from dietary histories, or by calculation made from such as slit-Biochemi

TABLE 3—Average biochemical values for Indian soldiers with positive physical findings compared with averages for all Indian troops

Physical finding	Number of cases	Serum ascorbic acid	Urinary ascorbic acid	Urinary thiamine	Urinary riboflavin
		Mg/100 ml	Mg/hr	Mcp/hr	Mcp/hr
Eyes					
Dryness	13	0.14	0.33	8.0	11.8
" "	100	0.18	0.35	10.0	12.7
" "	100	0.16	0.30	8.6	10.1
" "	20	0.19	0.41	11.3	14.9
" "	100	0.16	0.31	10.3	10.7
Cheilosis	60	0.17	0.37	11.6	11.0
Angular fissure	62	0.19	0.37	8.9	13.6
Poor oral hygiene	100	0.17	0.30	10.6	11.5
Good oral hygiene	100	0.19	0.42	11.1	10.4
Skin					
Follicular hyperkeratosis	49	0.13	0.38	10.8	13.3
Acneiform eruption	29	0.23	0.45	11.5	8.0
Seborrheic dermatitis	6	0.14	0.35	9.5	6.6
1,029 Indian troops		0.17	0.4	12.0	10.0

<sup>1</sup> Analysis of variance shows that the probability is less than 1 in 20 if at these averages differ by chance variation from the average of men not having the physical finding.

in the tissues. In natural environments, the interpretation of changes is difficult. A subject previously unsaturated may have had recent access to nutrients enough to raise his tissue concentration without restoring function or reversing pathological changes. Again, disturbance of nutrition is only one of many possible causes of functional impairment. Finally, a given pathological change, such as cheilosis, may result from one or many processes other than nutritional deficiency (Machella, 1942; Machella and McDonald, 1943). Pett (1945) emphasized another phase of the lack of correlation among chemistry, function, and pathology. Many characteristics of a population can be expressed in distribution curves. An individual subject provides a single point on each curve, but there is no valid reason to expect that measurements on him will always fall in the same portion of

optic disks. Since it is excessively uncommon for a single type of observation to be diagnostic for any specific disease, extreme caution should be used in interpreting data on the lower end of distribution.

but the distribution curves for the Indian troops had lower values than those for United States troops, and there were in Burma actual cases of ill health associated with positive clinical findings and poor biochemical states.

## II TROPICAL CLIMATOLOGY AND PHYSIOLOGY

## II TROPICAL CLIMATOLOGY AND PHYSIOLOGY

Figures represent (without minus with)

Physical finding	Average difference between men without and with the physical finding calculated individual islands			Step-test score
	Ascorbic acid	Thiamine	Riboflavin	
Figures of eyes	Avg./Isr +0.02	Avg./Isr +3.5	Avg./Isr +4	-10
Changes in opacity of sclera	+ .03	+ 4	+1	-6
Changes in opacity of cornea	+ .06	-1.6	-13	+3
Gross conjunctivitis	+ .05	-2.2	-3	+5
Pterygia	+ .14	+1.0	-6	+2
Pteroculae	- .02	- .9	-3	+5
Lips and mucous		+ 3	+2	+1
Gingivitis	+ .02	-3.8	-2	+4
Inflammation of dental margin	+ .03	+2.3	+5	+5
Swelling of interdental papillae	+ .04	+1.1	+5	-7
Swelling of gums	+ .05	+1.0		+2
Bad and poor oral hygiene	+ .11			
Skin		- .04	-1.2	
Full-blown hyperkeratosis		- .04	-1.1	
Acanthiform eruption				

1 Analysis of variance shows that the probability is less than 1 in 20 that these differences are due to random variations.

and poor oral hygiene, and (3) increased excretion of cross conjunctivitis and acniform eruption of these correlations was found with the highest levels of programs per ho

perils	+ 11	-1 2	(2)
inoculase	- 04	-1 3	
and pro in			
infectivity			
inflammation of dental margin			
inflammation of interdental papillae			
in swelling of interdental papillae			
in swelling of gums			
in swelling of gingiva			
in hair and poor oral hygiene			
in follicular hyperkeratosis			
in acneiform eruption			

Analysis of variance shows that the probability is less than 1 in 20 that these differences are due to random variables.

and poor oral hygiene, and (3) increased excretion of cross conjunctivitis and acneiform eruption. One of these correlations was found with the highest levels of grams per hour.

of riboflavin and poor oral hygiene, and (3) increased excretion of riboflavin and both gross conjunctivitis and acneiform eruption. Among the Indian troops, none of these correlations was found. In fact, poor oral hygiene was associated with the highest levels of excretion of riboflavin in Indian troops (15.5 micrograms per hour) which were still much lower than the lowest average figures found in United States troops (21 micrograms per hour). Two correlations among Indian troops were, slightly decreased serum ascorbic acid and follicular hyperkeratosis, and slightly increased serum ascorbic acid and acneiform eruption. The proliferative eye lesions described in the section on physical examination were not clearly associated with nutritional factors and we can advance no satisfactory hypothesis on their etiology.

The few correlations established were between small average differences in physiological interpretation was not apparent, and the few correlations established for diagnostic purposes in individual soldiers were not expected in a population suffering from a common disease. The expected correlation between the

The few correlations established were between small average differences, their physiological interpretation was not apparent, and they could not be used for diagnostic purposes in individual cases. A different set of results might be expected in a population suffering from florid nutritional disease. Lack of correlation between biochemical and clinical findings was reported by Milam and Anderson (1944) in North Carolina, by Riggs et al (1943) in Canada, and by Youmans et al (1942, 1943) in Tennessee, some of the many possible contributing causes for this situation were discussed by Smith (1944) and by Dann and Darby (1945). In a well-controlled laboratory experiment, imposition of a deficient diet is followed in order of chemical unsaturation of the tissues, by impairment of function and finally by definite pathological changes.

(Van Veen, 1942) The present data from the Pacific and Burma provide information on health adult male populations. United States troops were adequately fed when their daily nutrient intake included 100 grams of protein, two thirds of it animal, 0.7 gram of calcium, 20 milligrams of iron, 5,000 I U of vitamin A, 18 milligrams of

doing an efficient job in combat, construction and supply while receiving a ration which contained approximately 100 grams protein, one fourth of it animal, 2,000 I U of vitamin A, 25 milligrams of thiamine, 11 milligrams of riboflavin, 20 milligrams of niacin, and 40 milligrams of ascorbic acid. It is true that on the whole their

ponents of high vitamin intakes in the Tropics when a healthy adult population is in question. It was our impression that morale, fitness, and health of United States troops were not affected either adversely or beneficially by sporadic or regular use of vitamin pills. Neither can we support the proponents of a diminished protein intake in the Tropics. The arguments for and against this have been discussed by Lusk (1931), by Leitch (1930), by Nicholls (1938), and by Pitts et al (1944). United States troops voluntarily ate 100 or more grams of protein per day and adapted well to the heat. The Indians, with a lower intake of animal protein, had associated low levels of hemoglobin and serum protein.

Our observations support strongly the idea that caloric requirements are less in tropical than in temperate regions (Eijkman, 1924). Systematic surveys of American soldiers in Arctic, subarctic, mountain, temperate, tropical, and desert areas have yielded valuable evidence on this point. When ground troops are receiving abundant rations their voluntary caloric intake is inversely proportional to the mean temperature of the environment in which they live. The range was from about 2,900 calories per man per day in the desert to 4,900 calories per man per day in the Arctic for field troops with similar duties.

Systematic observations made on the energy expenditure for a given type of exertion in high, moderate, and low environmental temperatures showed clearly that caloric requirements are increasingly high as the temperatures decrease.

The best tactical group studied was the Gurkhas, whose fabulous performances in the war are well known. One hundred and forty soldiers from the 7/10 Gurkha Rifles were examined. Their performance in the test of physical stamina was very good indeed, with an average score of 92, which is considerably better than the 80 scored by a very good platoon of infantry in Canada (Kark et al, 1945), the 81 scored by the subjects from the United States 38th Infantry Division on Luzon, and the 85 scored by a well trained United States infantry battalion in Colorado. Physical examination revealed few positive physical findings, and those were not severe. Biochemical

age hemoglobin levels were somewhat lower.

We conclude that excellent physical fitness and tactical efficiency are compatible with what by ordinary North American standards are low levels of several important constituents of the body, notably riboflavin, serum protein, and ascorbic acid.

#### NUTRITIONAL REQUIREMENTS IN HOT CLIMATES

Nutritional deficiency diseases and poor nutrition are common in the Tropics. To this situation, at least three important factors (Van Veen, 1942) contribute: (1) Population density is high, (2) cheap staple foods, mainly cereals, tend to be limited in variety in any one region and to be unbalanced in important respects, and (3) disease rates are high. Among the best studies on diet and health in the Tropics are McCarrison's (1936). His main conclusions that typical regional, racial, and religious diets in India are very different in their ability to promote health have been substantiated by such other workers as Aykroyd (1940). Sikhs have the best civilian diet, and Madras is the worst. The Sikh diet provides an abundance of important nutrients and consists of atta (coarse wheat flour), milk, and milk products, such as butter, curd, and butter milk, dhal (legumes), vegetables, fruit, and meat. When McCarrison fed typical Indian diets to rats, growth and health were best on that of the Sikhs. Of all the castes and races studied by us, only the Sikhs had plasma protein levels in the same range as those of North American troops.

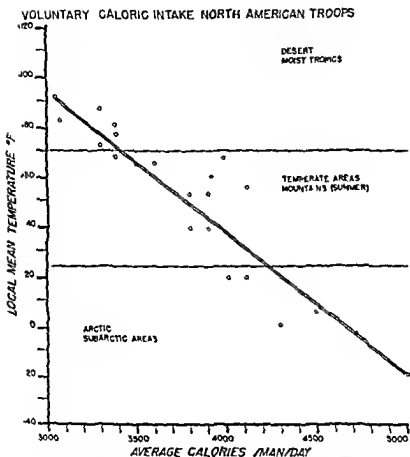
Animal experiments indicate that the effects of  
 summary:

observers agree that the caloric requirements of the rat are less in hot than in temperate climates.

There is much less certain knowledge on human nutritional requirements for life in the Tropics than on requirements elsewhere.

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AVERAGE FOR GROUPS OF FIFTY OR MORE MEN, WITH ABUNDANT FOOD SUPPLIES  
IN DIFFERENT PARTS OF THE WORLD

Figure 4.—Average voluntary caloric intake of groups of soldiers offered abundant food so that they could eat as much as desired. Ordinates: Local mean temperature of environment of group. Abscissae: Average caloric consumption per man per day



high urinary output of urine containing chloride. These patients cannot retain either salt or water, and I suggest that the continual striving of their bodies to conserve salt and water has partially exhausted the suprarenal and pituitary glands, through which the conservation is effected.

Soldiers on active service have little access to alcohol, but for many civilians an increase in fluid intake means automatically an increase in alcohol consumption. I am convinced this is important.

Dr Johnson mentioned the importance of sleep in his comparison between a mechanised and an animal transport company (made in a recent paper), the latter, from overwork and lack of sleep, were rapidly brought to the end of their tether. Dr Waterlow and I never saw such extremes, but we did notice the marked difference in condition, especially morale, between men working an early shift in Army work shops, rising at 4 a. m., having had only the hottest part of the night for sleep, and men on evening shift, who had a good

Anything that interferes  
hastens deterioration

ity and short temper, but  
after a tour of duty in the tropics, I would say that this aspect of deterioration is the cumulative effect of coping with indigenous inefficiency and of doing without amenities usually regarded as essential, for months on end.

deterioration, there is no such retreat. I would like to ask Dr Johnson in this connection what monthly variations in temperature and humidity he found in the Pacific.

time

Visiting various stations at different parts of the Belgian Congo, we find that a station, perhaps at approximately the same distance from the Equator in another portion of the Congo, is much more healthy than the place in which we are working. When we could not come home for a furlough on account of the war, we went to East Congo at about the same distance above the Equator but at a different elevation. There we found that the missionaries could stay as long as 15, 16, or 18 years without feeling the effects. We came back to our post somewhat refreshed but after about 6 months we were as tired as before.

We dread the beginning of the dry season, which is an ordeal in our part of the Congo, 4° north of the Equator. I believe that there

TAYLOR, G. F. and CHUTTANI, P. N. *Brit. M. J.* 1 800, 1945

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Canadian

Area 15

U. S. DEPT. OF AGRICULTURE  
Bureau of Tropical Plant and Animal Industry  
Washington, D. C.

b<sup>7</sup> Open Report No. 3—General Aspects of Tropical Fatigue as Seen in RAAF Ground Crew, Fourth Report of Field Investigation into the Incidence of Tropical Fatigue.

### ABSTRACT OF DISCUSSION

Dr W. S. S. LADELL (Nigeria), commentator Dr Johnson has shown that health and efficiency can be maintained indefinitely in tropical environments and that the well fed, properly housed man need not show tropical deterioration. Nevertheless some men, especially in isolated communities, do deteriorate physically and psychologically, whatever the climate. Dr Johnson believes it is always the same syndrome, I suggest that there are certain factors that impose a distinctive pattern upon tropical deterioration.

In the tropics it requires a constant conscious effort to drink enough to maintain fluid balance, but, as Dr John Waterlow and I showed in 1913, in many the effort fails, the 24 hour urine volume is less than 1,000 cubic centimeters, a small chronic water debt is contracted, shown by a fall in body weight, and there is haemoconcentration. Gross failure to drink, especially when coupled with salt deficiency, results in acute heat exhaustion, but another type of heat exhaustion, insidious in onset, is seen toward the end of the hot season, these patients are weak, listless, and noncooperative, on examination there is nothing definite beyond heated prickly heat in some cases and some times anidrosis, such men may be labelled malingerers. But biochemical examination shows a low serum chloride and an abnormally

## HUMAN CLIMATOLOGY AND TROPICAL SETTLEMENT

H C BAZETT, *Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania*

Valuable resources of lumber, minerals, and agricultural products in the Tropics remain undeveloped owing to our inadequate capacity to safeguard health. In the past, knowledge of tropical hygiene has grown simply from experience. Growth, though sure, has been slow. Now it is possible to apply experimental methods to the solution of

light construction and be well ventilated. From the point of view of the lowest initial cost for reasonable comfort, the assumption is correct. Yet low initial cost is no guarantee of economy, on the contrary,

structures of mere temporary value should light construction be utilized. These two views are diametrically opposed. Neither has been established incontrovertibly. Conversion from one system of construction to the other would be very expensive, both in time and money so that the relative merits of the two systems should be established as soon as possible by direct experiment. While at the present time the most urgent need, as in other parts of the world, is the provision

derived from unwarranted generalizations. The experience of temperate zones has been applied to the Tropics without critical judgment. Agricultural workers cannot be protected from the external conditions in which they are engaged. The intensity of the environment is tempered by the activity of the workers. The assumption that the intensity of the environment is tempered by the activity of the workers is false, because it assumes that conditions for cooling are the same both at rest and in exercise.

High external temperatures interfere with the quantity of muscular work which can be sustained but do not affect the intensity that can

are some real, definite things that take place out there that are explained in this military report. We try to keep mentally fit. I think there is something to this idea of the rigors of the tropics.

Dr C A Mills (United States) Since Dr Johnson brought name into his paper in connection with the use of the term "acclimatization", I think it would be quite appropriate here before this body to emphasize just what I would consider the meaning of the word "acclimatization". Since "acclimatization" is a process, the term is applicable to the individual as he reaches a level of response to his new environment in various of his physiological functions. The adaptation that was studied widely during the war years was an adaptation in the circulatory mechanism for the dissipation of body heat and it was found that there was a striking adaptation within 2 or 4 days. I do not like to have that called acclimatization. It is adaptation.

There is one other point that I think might well be mentioned and that is that Dr Johnson's studies were almost entirely on military forces on active duty. He did mention some British civilians in India. Well, even the British civilians in India fall into the category of the military forces here because they are getting vigorous exercise every day. We Americans don't intend to take vigorous exercise when we go into the tropics and I think that is one point that makes us more susceptible to tropical deterioration. It would seem that strenuous daily exercise to keep the metabolic machine steamed up would perhaps give the greatest protection against the tropical deterioration that comes on in four weeks to several months.

Dr H C Bazett (United States) Since I am due to talk in the next paper on the effects of tropical deterioration, I thought it might save me time if I picked up this quarrel with Dr Johnson. I don't quarrel with his observations, but with his generalization. I do not think one can defend tropical deterioration in the kind of test he is talking about.

Let me take an example. Sleepiness can be one of the very important factors. I once did an experiment lasting 40 hours. In the last 28 hours we were taking samples from ourselves and animals every hour or so. We never had more than 5 hours sitting down. Toward the end of that time when I bled anybody else, he said "What the flunkety, blankety are you coming in here to take blood when you don't know where the thing is!" When I was a subject, I said the same thing. And yet, when the sun came up, we behaved like more reasonable human beings, and were able to bleed more easily. I did physical tests like pulse rate responses, exercises holding my breath, and so on, and every time I did better than I did before in any normal condition. Now, Dr Johnson, if you don't believe I was inefficient come and let me bleed you under three conditions and you will agree

tatively in terms of its reciprocal, that is its thermal insulation (referred to the conditions in this and other normal subjects is probably about 0.2 Clo (the lower value given for the fourth temperature condition of the table is probably dependent on absence of equilibrium) This figure implies that for a person at rest environmental conditions for comfort should be such as to allow adequate removal of heat at surface temperatures not exceeding  $35.5^{\circ}\text{C}$  Any rise of surface temperature above this level necessitates an undesirable increase in deep body temperature above  $37^{\circ}\text{C}$  ( $98.6^{\circ}\text{F}$ ), which may decrease both comfort and efficiency

tion is demonstrated in values for tissue insulation, which are only about one third as great as the minimal value attainable at rest Surface temperatures also remain low, owing partly to increased air movement but mainly to the utilization of evaporative heat loss At the temperature levels concerned, air can remove much more heat per unit volume by absorbing water vapor than it can by being directly warmed The adjustment of external heat loss is, therefore, possible provided that the air can accept adequate water vapor, even though the heat acceptance may be negative

The table allows the relative strains of rest and work to be compared At the two lowest temperatures no great strain is imposed

This is indicated by a rise in rectal temperature at rest but not at work, decreasing the differences between the two conditions At the next to highest temperature the strains have again become about equal since the water acceptance of the air is near the limit for this degree of work Actually the evaporative loss was derived from 637 milliliters of sweat only, while an additional 540 milliliters of sweat, though formed, was ineffective from failure to evaporate At the highest temperature the strain during work became the greater, for the heat load was too large for the water acceptance of the air in the face of a considerable negative heat acceptance

such interference is unnecessary and, by even more, dismissed, without trial, as either useless or impracticable.

The physiology of a man during exercise is very different from that at rest. At rest some 60 percent of the heat produced is generated in central tissues and only some 15 to 20 percent in the muscles of the limbs, yet much of the heat loss is from the limbs, which serve as radiators. Heat transfer from the trunk to the limbs is an important factor. On the contrary, in muscular work most of the heat is generated in the muscles and much of it in the limbs. Transport difficulties are eased. Air movement across the skin is accentuated by the movements, so that both convective and evaporative heat loss from the surface to the environment are aided. Muscle work is also accomplished more readily at higher muscle temperatures, and body temperature is regulated at higher levels during work. Increases in early implying inadequate heat loss (Nielsen, 1938).

Some data on a single subject selected from those reported by Robinson et al (1944) are given in table 1 to illustrate these points by specific instances. Temperatures are given in the usual terms of dry bulb readings and relative humidities, though the latter is very misleading for physiological work. Fully saturated air is able to take up considerable quantities of moisture, when it is warmed by contact with the skin. Normally the maximal temperature for the body surface under conditions of comfort at rest is  $36^{\circ}\text{C}$ , at which temperature saturated air has a vapor tension of 41 mm. The capacity of the air to accept moisture from the skin may therefore be expressed in terms of the deficit of its vapor tension below 41 mm, and this difference may be called its heat acceptance value. Similarly the environment may be said to have a heat acceptance value to the extent to which its temperature is below this assumed value of  $36^{\circ}\text{C}$ . These values are indicated in the table. By use of suitable constants (varying with wind movement, etc.) the actual heat losses may be calculated, but such constants are as yet not well-established. They have been particularly developed by scientists in the office of the Quarter Master General (Ionides et al, 1945) and should have real value.

The subject had a surface area of  $1.77\text{m}^2$ . His energy exchange at rest was  $46\text{ kcal/m}^2/\text{hr}$  and during level walking  $120\text{ kcal/m}^2/\text{hr}$ . In both cases most of this was dissipated as heat. At rest heat transfer from the trunk to the surface could not be maintained as temperature differential of about  $15^{\circ}\text{C}$  increased as the environment had also to increase. The thermal conductivity

TABLE 1—*Jungle uniform Subject L G—Data of S Robinson*

REST—Wind 53 m/mhnts										WALKING—5 km/hr (2.8 miles) Wind 53 m/mhnts									
Temperature air °C °F	Humid, Percent saturation	Heat ac- ceptance Water ac- ceptance	Heat balance		Temperature, rectal surface °C	Temperature, differ- tials humid (Cio)	Pulse rate	Temperature air °C °F	Humid percent saturation	Heat ac- ceptance Water ac- ceptance	Heat balance		Temperature, rectal surface °C	Temperature, differ- tials humid (Cio)	Pulse rate				
			Prod kg cal/ M/hr	Loss evap Non evap							Prod kg cal/ M/hr	Loss evap Non evap							
21.9 71.4	27	14 39	+46	-4 -42	36.0 32.4	3.8 4.3	55	21.8 71.2	31	14 38	+130	-54 -76	37.2 33.1	4.1 18	90				
28.1 82.6	28	8 37	+40	-15 -31	35.1 34.6	1.6 1.9	58	28.4 83.1	30	8 33	+130	-105 -25	37.35 33.3	3.55 16	87				
37.7 99.9	41	-2 34	+46	-100 +34	34.8 34.7	2.1 2.5	64	37.7 99.8	42	-2 23	+130	-180 +59	37.3 34.3	2.6 11	87				
37.9 100.3	64	-2 18	+46	-20 +14	37.2 36.0	1.2 1.5	77	37.9 100.2	62	-2 14	+130	-202 +9	38.0 36.35	1.65 0.7	107				
43.0 113.1	43	-9 14	+46	-164 +118	37.35 35.9	1.45 1.8	88	43.3 113.6	41	-3 14	+130	(1-400) (+320)	38.9 37.7	1.2 0.3	130				

‡ In this experiment the sweat loss was measured, but satisfactory estimates of the amount of sweat loss were not obtained.

\* In this experiment the sweat loss was measured, but satisfactory estimates of the amount evaporated were not obtained. The values given are maximal rather than real.

The claim that the mild increases in rectal temperatures observed at rest were accompanied by inefficiency for mental tasks rests on other evidence obtained under similar conditions, particularly in England

those cited in table 1, the degree of disability may be estimated approximately for the conditions cited by interpolation of a graph given by Mackworth. The average increase in errors would be about 20 percent for the temperature of  $37.7^{\circ}\text{C}$  ( $99.9^{\circ}\text{F}$ ) and 41 percent

importance, he is also inefficient, and this is of immense importance. The cause of such effects is not known. A rise of deep body tempera

Emphasis has been placed on these simple data to demonstrate how complicated is the situation and how easy it would be to draw false

reasonable cost in time or money, except by systematic experiments on an international research at . . . However,

study of this field, arranged to supplement the public resources already made available to these laboratories by UNESCO, might be amply repaid

sequent inefficiency. Later the submarines were air-conditioned, and



delphia for 1947 is indicated in figure 1. The low temperature used as a standard allows for the heat contributed by men and cooking. While a normal temperature is usually considered about  $22^{\circ}\text{C}$  ( $72^{\circ}\text{F}$ ), the actual temperatures used commonly exceed  $24^{\circ}\text{C}$  ( $75^{\circ}\text{F}$ ), even though indoor work could certainly be accomplished at temperatures as low as  $15^{\circ}\text{C}$  ( $59^{\circ}\text{F}$ ).

The higher comfortable

only add to our pleasure but to our efficiency.

In a tropical climate the cooling load should be similarly estimated. The cooling need not exceed reduction below  $25.5^{\circ}\text{C}$  ( $78^{\circ}\text{F}$ ) at the most, for experience shows that acclimatized resting subjects are comfortable at such a level, even if the air is saturated. Nor would water necessarily have to be removed, for even in humid tropics the water vapor tension rarely consistently exceeds 24 mm, the saturation value for this temperature. Allowance has to be made for the heat of human metabolism, and consequently the load may be calculated for the excess above  $23^{\circ}\text{C}$  ( $73^{\circ}\text{F}$ ). The average monthly cooling load for Bombay calculated on such a basis is also shown in figure 1. The yearly estimate is  $1,340^{\circ}\text{C}$  days or  $2,170^{\circ}\text{F}$  days, which is considerably less than that for heating houses in Philadelphia or New York (some  $3,000^{\circ}\text{C}$  days). Though the cost of reducing humidity has not been included, the estimate is probably not grossly in error, for absolute humidity would rarely have to be altered in a theoretically fully efficient system.

The energy cost of cooling in a tropical city should not, therefore, be prohibitive.

ters of civilization.

established  
into cooler  
climates in

door climate. Without such development, civilization could not have flourished in the Northern States or in Canada, or be now beginning to spread in Russia. It is possible that air conditioning may have in the future as great effects on cultural development in the Tropics, as has central heating in colder climates.

Information cannot be obtained with sufficient accuracy and speed by the trial and error method of experience or by historical analysis. Toynbee would assign cultural developments of the past to the reactions to trouble and would assign to climatic conditions an important role as a stimulus. Yet history may fail to recognize factors which

the crews became far fitter than those of the surface vessels, even though such surface crews were believed to deteriorate mainly from psychological causes. The economic value of comfort through its association with real health and efficiency was demonstrated incontrovertibly.

Proof is needed, and experimental proof would be less expensive than would ill-controlled experience, whether such air-conditioning, available in homes or public buildings, would justify its cost, provided that the users had to be exposed by day to outdoor heat. There is little doubt that critical observers, familiar with tropical areas where nights are both hot and humid, would agree that provision of good sleeping conditions is of primary importance for efficiency. Considering tendencies to overbreeding commonly found in tropical areas, it would also be important to discover whether air-conditioning of homes would increase or decrease the birth rate.

The average cost of a good night's sleep in a room with air conditioning is about 10¢ per person per night.

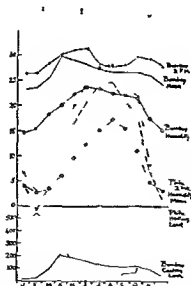


Figure 1.—The normal average temperature conditions in Bombay are compared with the average values for Philadelphia in 1947. Months of the year are plotted as abscissae. In the upper graphs average monthly temperatures are indicated in °C. and absolute humidities in millimeters Hg vapor tension. Continuous lines are used for Bombay and dotted lines for Philadelphia. Measurements made at 1.30 or 2 p.m. are indicated by crosses, and humidity values at the same time by open circles. In the lower graphs the monthly cooling load (in °C.-days) for Bombay is compared with the heating load for Philadelphia.

activities and also on the genetic variability of strains, a phenomenon presumably related to virulence

The change from a drug susceptible to a drug resistant strain is a remarkable phenomenon in genetics. The most commonly accepted explanation is that all bacillary populations are mixtures of natively resistant and susceptible strains, the latter being predominant prior to treatment, and that a drug suppressing the latter permits the others to survive and become preponderant by the process of natural selection.

## I

to this particular character

The numerous investigations of the chemical composition of the tubercle bacillus and its metabolic products, when pursued further, may throw light on drug resistance and drug susceptibility. The studies of Anderson on the lipids of the tubercle bacilli and Seibert on the proteins and carbohydrates of tuberculin show a variability in composition of products from different lots of bacilli that cannot be predicted on the basis of the usual known factors of culture, such as temperature and duration of growth. These variations are sufficient

supposedly pure strains has been well shown by the studies of Petroff, Dubos, and their associates, who have described variations in the colonial morphology and character of small aggregations of bacilli which are correlated with virulence. Such experiences once considered annoying in the search for stable strains, may be fruitful in explaining why some strains are more virulent than others and why virulence of a supposedly stable strain may change.

## IMMUNOLOGY

Current and recent studies have thrown light on both native and acquired immunity. In human beings the significance of native resistance to tuberculosis has not been easy to evaluate because of the great practical difficulty in separating inherent and environmental factors. Nevertheless fundamental racial differences in resistance, and hereditary variations as shown by studies of identical and non-identical twins and family groups, seem generally accepted today, as indicating that resistance and susceptibility to tuberculosis depend on certain inherent qualities, hereditarily transmissible (Diehl and Verschuier, Kallman, Puffer)

of resistance and susceptibility may be maintained by appropriate

may be obvious to contemporary observers. Thus many Scottish youths, and also American youth brought up in northern county districts do conspicuously well in competition in universities. Their success might be attributed to the difficulties overcome in their hard climates. An alternative explanation might be that in such surroundings

work should be made preferable to those for other pastimes. Contemporary study of all the factors, by experimental and observational methods is needed to guide contemporary growth.

To promote culture in the Tropics, air conditioning should be applied probably at first to the homes and particularly to sleeping quarters, and hospitals secondly to libraries, schools and colleges

Tropics have accomplished in the past remarkable feats in spite of a hard climate. Who knows what they might attain with reduced handicaps?

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#### ABSTRACT OF DISCUSSION

Dr M. NARASIMHA RAO (India), commentator. It is really commendable that such international organizations as UNESCO are contemplating research work on how best the physical environment in the tropics could be improved on scientific lines. Perhaps as Professor Bazett suggests, more has to be done than organizing a single unit in a single place. Brazil. More units in more places have to be started. India provides a variety of climates for research and I can assure on behalf of the Commission that it is ready to co-operate in such work from the

One of the most significant findings with respect to allergy is its passive transfer. The temporary maintenance of allergic sensitivity in tissue cultures from sensitized animals (Rich and Lewis, Aronson, and others) suggested that such transfer should be possible. Moreover, Moen has shown that the sensitivity to tuberculin of cells in tissue culture can be maintained in serial transplants, a fact suggesting that the allergizing mechanism resides in the cells and is not dependent on humoral factors. The more recent studies of Chase and Kirchheimer and Weiser appear to have settled the problem. Exudate cells, from a guinea pig sensitized with dead tubercle bacilli, when injected into a normal pig, make the latter temporarily sensitive to tuberculin. Since this effect is secured with well washed cells, the implication is that the elements responsible for hypersensitivity are within the cells of inflammation. Presumably when these disintegrate in the new host, the latter's phagocytic cells take them up, the animal remaining allergic until they disappear.

Studies on the serum of patients with tuberculosis (Seibert) show

body but may also be related to serum immunological characters, particularly in the case of gamma globulin in minimal active tuberculosis.

### PATHIOGENESIS

New facts obtained in recent years help to explain the pathogenesis

infection, although neither then nor now is it clear whether that re infection is usually exogenous or endogenous.

breeding, but also they give definite indications of the mechanism of resistance. In some respects natively resistant animals behave like animals with artificially induced immunity. In each case, the response

infected

It is not always possible to differentiate clearly between inherent and environmental influences. Lurie and others have shown that the sex hormones, which are presumably inherent constitutional factors, mediate the response characteristic of resistance or susceptibility, estrogen retarding the spread of the disease and luteinizing hormones enhancing it. Nutritional factors which may be considered environmental, modify constitution also, perhaps they do this only temporarily, but while they are in operation they play a part analogous to the hormones. The inhibitory effect of ascorbic acid on the tuberculin reaction (Steinbach) is an example. The role of nutrition has never been accurately evaluated, by those who stress the importance of contagion it has even been doubted that it has a role. However, a small number of controlled studies by McConkey, Getz, and others support the view that vitamin A, ascorbic acid and protein are protective in some measure against the progress of tuberculosis.

That resistance to tuberculosis can be heightened by artificial stimulation, as by infection with a tubercle bacillus of low virulence or by the inoculation of dead bacilli, has long been known. However, the world has been slow to accept vaccination with either dead or live bacilli as a practical procedure. Recent years, however have witnessed

such as hospital personnel

Relatively little attention has been devoted to the nature of the immunity	the character of
the vaccination	with different
strains of	acid fast bacilli
have shown	

Immunity in tuberculosis has a recognized although not clearly defined relation to allergy. The two conditions can be separated by

stimulate allergy without raising resistance. Confirmation of these reports will be important.

the vast difference in the prognosis of cases with and without gross excavation. Once excavation has occurred, a vicious cycle is established, with copious reseeded of the lung and excavation. Follow up studies on tuberculous cases in different stages always emphasize

..

cate that its prognosis is excellent. These studies, based on mass X ray surveys of unselected populations, with no history of previous clinical tuberculosis, uncover more cases of inactive than of active pulmonary infiltration. This situation will presumably change when surveys are performed on the same population more frequently. When that time comes, relatively more fresh infiltrations will be discovered and follow up studies will perhaps disclose the individual differences in constitution, environment, and behavior affecting progression of infection.

An important outgrowth of mass surveys that has a bearing on the problem just discussed is the recognition of nontuberculous chronic pulmonary infiltrations with an appearance similar to that of pulmonary tuberculosis. Evidently some of these, including histoplasmosis (Palmer, Christie, and others) may proceed to calcification not unlike that of healed childhood type tuberculosis. These studies help to explain the frequent occurrence of healed calcified infiltration in subjects not reacting to tuberculin. They do not indicate that a calcified lesion in the lung in the absence of a positive tuberculin re-

disappear

In this connection, it should be pointed out that it is still impossible, in spite of a growing conviction of its specificity, to find a dose of tuberculin which will detect all positive reactors and not cause a tuberculin like reaction of nonspecific character in some individuals. A compromise dose, with slight inaccuracy in each respect, can, however be established.

#### EPIDEMIOLOGY

The mass X ray surveys based on the photofluorographic method introduced by De Abreu have led to a new understanding of the frequency of tuberculous infection in adult life. Allowing for uncertainties in diagnosis it still appears that the figures once accepted for the frequency of pulmonary tuberculous infection must be at least

culin positive state that its nature as a first infection can be recognized

Opinion is divided about the relative seriousness of first infection

In the United States, on the other hand, first infection in adults does not carry such a serious prognosis. The great majority of nurses and medical students, in whom such infections have been observed, make complete and lasting recoveries. It should be noted, however, that most of them take rest treatment and avoid factors which might favor progression.

In one respect, the development is quite unlike that of first infection tuberculosis in childhood. The first manifestation of primary infection in young adults is very frequently pleurisy with effusion. This phenomenon has been observed in many cases of primary infection.

(Adamson) Pleurisy with effusion has always been looked upon as an allergic manifestation, presumably the pleural infection leading to the effusion in such cases occurs in the early period of high degree allergy following first infection.

The pathogenesis of pulmonary excavation remains unsettled. The analogy to a Koch phenomenon is still mentioned but does not explain cavity formation in first infection in young adults. To be sure, the

Lurie has shown that small, controlled, first infection causes localized, fibro ulcerative tuberculosis in rabbits of susceptible stocks. The actual mechanism of excavation is a puzzle. There seems reason to

ing in the pathogenesis of phthisis is an appreciation of the importance of endobronchial tuberculosis. This common condition is often responsible for the continuing excretion of tubercle bacilli and is intimately related to cavity formation and the effectiveness of collapse therapy. A stenosis of endobronchial

one of the most striking results of streptomycin therapy is in the arrest of early endobronchial lesions, a measure likely to be effective in preventing the formation of tension cavities.

In connection with cavity formation, reference should be made to



It has many of the characters of phthisis, including localization and tendency to cavity formation. In the United States, at least, under

with pleural effusion. Endobronchial tuberculosis plays an important

the epidemiology of  
 tendency of spontaneous  
 healing of pulmonary lesions of minimal extent, as made evident by  
 mass X ray surveys, and an improved correlation of the time of  
 tuberculous infection with that of development of the disease. The  
 first 2 years of the first infection in adults are recognized as a period of  
 special hazard. The general postponement of first infection is one  
 factor, at least, in the current shift of tuberculosis mortality toward  
 the later years of life.

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years of life shows how commonly tuberculous pulmonary infiltrations of the type once called reinfection heal spontaneously

opinion was accepted by clinicians that tuberculosis rarely developed after the age of 40 in a person whose lungs were free from pulmonary tuberculous infiltration before that age. With the far greater frequency of X-ray examination in all ages, it is now clear that new and

posed groups such as medical students, nurses, and hospital interns in which serial records of tuberculin tests are kept show that a high proportion of the clinical tuberculosis that does develop in such groups becomes evident within 2 years after first evidence of tuberculous infection in the form of a positive tuberculin test. All such facts are obviously of great importance for planned tuberculosis control.

#### SUMMARY

Advances have been made in recent years in the bacteriology, immunology, pathology, and epidemiology of tuberculosis. The Dubos method of rapid subsurface culture of the tubercle bacillus has proved useful for studies on virulence and genetic variation. The phenomenon of acquired resistance to growth suppressive drugs like streptomycin is throwing light on the metabolism and genetics of the organism.

Hereditary variation in resistance to tuberculosis in man and animals, based on inherent constitutional factors, is a fact generally accepted. The constitutional factors concerned are in part hormonal. The principal determinative factor in resistant and susceptible animals when infected by tubercle bacilli appears to be the rapidity and intensity of reaction at the site of inoculation. In this respect there is an analogy between natively resistant animals and animals with acquired immunity. A relationship with allergy is to be recognized even though allergy and immunity are separable. The passive transfer of allergy has been achieved.

Under modern conditions of infrequency of exposure, first infection with tuberculosis commonly occurs in young adults. Opinions vary in regard to its gravity as compared with first infection in childhood.

the vaccination of some 200,000 persons strain No 292 from 1926 to 1932, strain No 450-S1, from 1933 to 1937, strain No 568-S1, from 1938 up to now

We have followed practically to the letter Calmette's original technique (18) for the maintenance of the strains and the preparation of emulsions from the 19 day old veils on Sauton's medium. We have systematically avoided using any other culture medium as well as changing brands of ingredients. Our vaccine came as directly as possible from cultures on bile.

## RESULTS

### STABILITY OF THE POSITIVE CHARACTERS OF ATTENUATION

#### 1 *A Word on the Constant Cultural Particulars Since 1926 of Our Three Successive BCG Strains, Nos 292, 450-S1, and 568-S1*

The state of being alive of BCG is the first condition of its activity; hence our main concern must be to secure young and healthy cultures. We believe that, in our emulsions, the number of living cells is constant because the technique of culture, the rate of transplantation, and the preparation of the vaccine are always the same, those of Calmette as explained above, and because the cultures and the veils are prepared in the same manner as in 1926. In 1926, the culture was on Sauton's medium.

Moreover, the practice of preparing BCG in quantities never inferior to 1 liter (the equivalent of a 5 gram veil on Sauton's) and the use of a 5 gram weighted culture, the small chances of error in the preparation of the emulsions, and to the use of the vaccine in man.

#### 2 *Similar Potency of Our BCG Tuberculin in 1935-36-37, as Compared With That of Calmette's Tuberculin in 1926*

Graph 1 shows that for the years 1935, 1936, 1937 (strain 450-S1) the tuberculin prepared with our BCG have behaved almost identically toward the standard. Calmette also, in 1926 (19) compared to the standard tuberculin those tuberculin derived from BCG culture on Sauton's. Confronting our graphical results with his, we conclude that BCG, in our laboratories and at periods of time remote from Calmette's experiments, has produced tuberculin the activity of which is fairly constant and equal to that of tuberculin prepared by the French discoverer.

## SOME EXPERIMENTAL AND CLINICAL OBSERVATIONS ON THE STABILITY OF BCG VACCINE

ARMAND FRAPPIER, *Director, Institute of Microbiology and Hygiene  
and School of Hygiene, University of Montreal, P. Q., Canada*

After Romer (1), who postulates that in tuberculosis the amount of protection is directly related to the virulence of primary infection, a few authors (Kraus (2, 3), Selter and Blumentberg (4), Gay (5)) have put forth the hypothesis that BCG, having undergone since the earlier work of Calmette a great number of extra passages on bile potato, would have still decreased in virulence and consequently in activity. Actually, Zeyland and Piasecka Zeyland (6), while no longer succeeding in recuperating BCG in cultures from organs of subjects vaccinated 5 or 6 months before, did think of a decrease in the vitality of BCG. K. A. Jensen (7), having experimentally observed certain variations in the power to produce more or less important and persistent intradermal lesions, as well as a more or less early and in

various intervals

If BCG were decreasing in its immunizing capacity, we would be compelled, as suggested by Gay (5) and Zeyland and Piasecka Zey

studied the stability of the antigenic and sensitizing properties of BCG; Saenz (13) and Saenz and Costil (14), the recuperation of

The aim of this communication is to show that in Canada BCG seems not to have undergone those weakenings or variations that,

biology and Hygiene of the University of Montreal through its BCG Vaccination Service prepares the vaccine for the needs of the whole country. Three successive BCG strains have been used in Canada for

including 16 percent of abscesses which opened spontaneously or had to be punctured

With the intradermal method our figures are higher than those of most authors, whereas with the subcutaneous method they are much lower

We have compiled our data and those of other authors concerning the incidence of local lesions due to BCG, for the sole purpose of proving the impossibility of arguing from these findings for or against the stability of BCG in its toxic properties

#### 4 *Periodical and Comparative Studies of the Allergizing Activity of BCG as Shown by the Development of Tuberculin Allergy*

(a) *In the BCG-vaccinated animal*—With Fredette (46, 47) I have studied for a number of years the development of tuberculin allergy in BCG vaccinated guinea pigs with different doses and by different methods

Graph 2 offers a comparison between one of our typical curves, picturing the mean sensitization of one lot of 10 guinea pigs inoculated with a strain of BCG during 1934-36 and the same curve obtained by Boquet's strain

Boquet's strain was then comparable in every respect with that obtained by us. Other similar curves have been built from our results in guinea pigs inoculated with different BCG emulsions during the years 1934-36. Thus our BCG strains seem not to part from those of the Institut Pasteur in regard to their sensitizing properties in animals

(b) *In BCG vaccinated humans—fairly comparable results of intradermal BCG vaccination*—A study of the incidence of BCG vaccination in humans, with a dose of 1 milligram

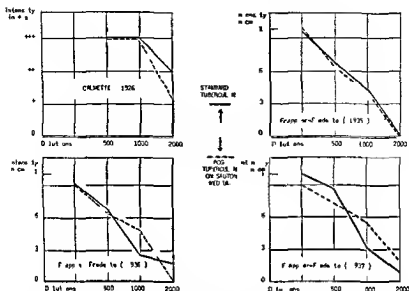
Having noticed periodical or casual variations in the percentage of positivity obtained during a few years, they conclude that BCG varies in its so called virulence, or, better told, its vitality and toxicity. These differences, as reported by Wing and other authors, are not important and range between 10 and 20 percent

It is important to

To be noted also is the fact that the incidence of BCG vaccination is increasing in the tropics, corresponding to the

with about 1 million strains, record a

tuberculin allergy incidence of at least 80 percent or more (38, 40, 50-52, 54, 111, 115, 116, 87-92) 6 weeks and 1 year after vaccination, testing with strong doses of tuberculin (1 milligram)



Graph 1 Comparative potency of BCG tuberculin against a standard tuberculin  
(A Frappier and V Fredette)

3 Periodical and Comparative Studies of the Toxicity of BCG, as Shown by the Incidence and Persistence of Lesions Produced by BCG in Animal and Man

(a) Peritoneal lesions in the guinea pig—From 1933 (70) we have

to BCG. However, Calmette, Boquet and Nègre (20), as well as other authors later on (17, 21-29) seem not to have neatly established the rate of incidence of those peritoneal reactions. A J Togounova (30) remarked in 1929 a great irregularity of those lesions in guinea pigs killed at the same time.

In table 1 we have compared (1) the periodical incidence of omental nodules in two groups of guinea pigs inoculated with 10 milligrams of BCG (strain 450-S1) at two periods of time (1933-1935 and 1935-

other vaccinated with strain 568-S1 and observed from 1946 to 1948.

In 1937, Calmette asserted that those lesions happened in about 30 percent of the cases when 10 milligram doses of BCG were used. Our findings with a 10 milligram dose fairly corroborate Calmette's contention. With the 5 milligram dose, nothing authorizes us, from

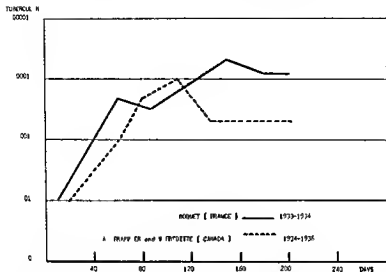
Our results for orally vaccinated subjects at the third and sixth months compare with those of de Assis and de Carvalho (53) for the period 1927-1932 from a great number of vaccinated subjects tested with 1 milligram and with those of de Assis (54) in 1928-1939, that is, 87 percent against 78 percent and 83.7 percent at the third month and 93 percent against 85.5 percent and 89.7 percent at the sixth. They also compare with those of Gomez Ullate (55), published in 1945 from children vaccinated with 30 milligrams, that is 87 percent against 80 percent at the third month after vaccination.

By the multiple puncture method (36 punctures; 15 milligrams BCG concentration per cubic centimeter in adolescents) Frappier and Landry (56) have obtained in 1944, between 1 and 2 months after vaccination, 95 percent of positive results, which are sharply similar to Rosenthal's (57), i. e., 96.6 percent in 1948 in new-born, to Bal

TABLE 3.—Comparative incidence of tuberculin allergy periodically induced in groups of human BCG vaccinated subjects, 1936-47

(A FRAPPIER AND L. FORTE)

Groups—Year of vaccinations	Number of BCG strains—Year	Number of subjects tested	Periods of testing in months—Percent of positivity				
			6	12	24	60	72
Oral method of vaccination—Newborn—30 milligrams of BCG—Tested tuberculin P P D 4 milligrams I D							
A 1 (1939) A-2 (1939) A (1940)	508-81 (1937)	218	100	99	99	—	—
	84-82 (1937)	73	—	—	100	—	—
	808-81 (1937)	54	100	—	100	—	—
	Controls not vaccinated—Brothers and sisters of vaccinated (1 to 6 years)	—	—	—	—	—	—
B (1941) A-2 (1939) A (1940) B 2 (1938)	—	85	26	—	24	30	—
	—	78	—	24	42	—	—
	—	64	33	—	21	—	—
	—	190	—	34	29	—	34



Graph 2 Comparative development of tuberculin allergy in groups of guinea pigs vaccinated with BCG and observed by two different authors (A Frappier and V Fredette)

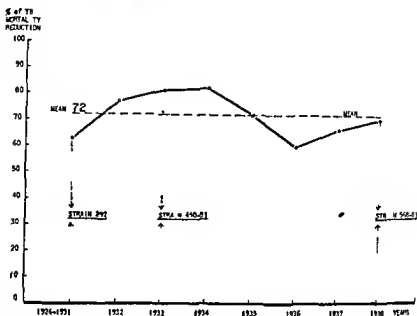
More constant results are even obtained, as further explained, if comparisons are made with persons vaccinated by the scarification or multiple puncture methods. As early as the second month, an allergy of nearly 100 percent is obtained (41, 56-58, 61, 93-100).

(c) *Human results in Montreal—yearly incidence rates of tuberculin allergy—comparison with results of foreign authors*—We have studied the development of allergy in subjects vaccinated by the oral, subcutaneous and scarification methods, while we followed the same in control subjects, siblings of vaccinated subjects in certain groups (table 3). First the subjects were always tested with P P D tuberculin, first dose. Those found negative were at once reinoculated with the strong 5 milligram dose. Controls were aged 1 to 6 years at

allergy may then fairly be attributed to BCG. In none of those families were known cases of tuberculosis reported by the social survey.

Taking into account what has been said above in connection with the non vaccinated controls, siblings of the former, one cannot help admitting a fairly commendable stability in the allergizing power of BCG, either oral or subcutaneous, for the period these studies have lasted.





Graph 3.—Early variations of protection for the first year of life as compared with the mean for a period of 10 years—BCG oral vaccination 30 milligrams three successive strains (J A Baudouin and A Frappier)

TABLE 4—Guinea pig protection obtained with 2 strains of BCG at 9 years interval

[A FRAPPIER AND V FREDETTE]

Year of experiments	Number of BCG strains	Number of guinea pigs		Mode of vaccination—Dosage	Virulent dose	Average survival (days)		TB degree (average)	
		Vaccinated	Controls			Vaccinated	Controls	Vaccinated	Controls
1936	450-S1	20	20	30 milligrams S O	0.0001 milligram S O	237	148	II	III
1945	408-S1	22	25	30 milligrams S O	0.0001 milligram S O	218	168	II	II

Taking the mean results we find that the ratios become 1.452 in favor of orally vaccinated persons, and 1.452 in favor of intradermally vaccinated subjects. For this latter method of vaccination, the mean protection ratio against morbidity lies at 1.4.

We shall now see how closely the results obtained in Canada fit with those averages.

studies ever carried on concerning the value of BCG

So, from table 5, it may be inferred that the protection given by the

95 percent of positive results, and 100 percent after 3 months, whereas Nègre and Bretey (62), in 1940, using a nearly equal dose of BCG in adults and testing them with the Von Pirquet test, have obtained 85 percent after a month and 100 percent after 2

Thus it seems reasonable to conclude that, in our hands, and with two different strains BCG has maintained definitely stable its allergizing properties and that our results agree satisfactorily with those of other authors at different times and in different countries

5 *Periodical Comparative Studies on the Immunizing Activity of BCG as Shown by the Development of Tuberculin Allergy*

(a) *In the guinea pig*—It comes out of the works of Calmette (63) and of a few earlier authors (64, 65) who have corroborated him, that, in guinea pigs vaccinated with a strong dose of BCG at one time and challenged along with controls with mild doses (0.5 milligram) of virulent bacilli, the protection shows an average survival of 3 to 4 months over that of controls

When the virulent dose is very small, the survival, and consequently the resistance, are lengthened

Table 4 summarizes two typical experiments conducted in our laboratories, one in 1936, with our strain 450-S1, the other in 1945, with strain No 568-S1. Our animals were vaccinated with 30 milligrams BCG and challenged with a 0.1 milligram virulent dose. The average survival of the controls after the virulent inoculation remained the same in both experiments, 146 and 148 days. The average survival of vaccinated animals was 237 and 216 days. The severity of tuberculosis was estimated at III and II for the controls respectively in both experiments, as compared with II for the two lots of vaccinated animals.

The total survival of all our vaccinated guinea pigs lies, therefore, between the seventh and eighth months, while that of our controls is the fourth and the fifth after the virulence test. These results are still within the limits of those of Calmette and earlier authors as mentioned above. On the other hand, at a 9 year interval the im

(b) *In human beings*—(1) *Average protection against morbidity and mortality, reported by Canadian authors, as compared with the mean protection calculated from foreign results*—From the present point of view, that of studying the stability of the immunizing power of our BCG for man, it may perhaps be permitted to look, with precaution, to see if a given result in Canada approximates the average protection found by foreign authors

BCG, there could happen *in vivo* within certain limits a more or less great number of bacterial cell divisions, keeping up more or less strongly and durably the action of their own toxicity. Then, if the variations of the toxic characters of BCG were proven, they would explain better, through limited ups and downs of that vitality, expressed by a relative and limited number of generations *in vivo*, than by variations of the so called virulence.

### SUMMARY

1 Three successive BCG strains have been studied in Canada, from 1926 up to now, regarding the periodical and comparative stability of BCG.

2 The results in comparative tables and graphs show that Canadian strains have not undergone important variations, if any. Canadian authors technique, biology, reported another and comparable to the average from foreign authors.

### Canada

TABLE 5.—Protection obtained by Canadian authors as compared with a mean established from foreign authors

[A. FRAPPIER AND R. GUY]

	Oral vaccination— Ratio of TB mor- tality—vaccinated controls	Intradermal—Ratio TB morbidity— vaccinated controls	Vaccination—Ratio TB mortality— vaccinated controls
Authors (mean)...	1 1.88	1 4	1 4.52
J. A. Baudouin	1 3.20	1 4.85	—
R. G. Ferguson		1 4.27	1 4.50
		1 5.03	

TABLE 6.—Protection obtained in humans with 2 strains of BCG at respective periods R. G. Ferguson (intradermal vaccination 0.20 milligrams)

Description of groups	Period of—	Number of BCG	Number of subjects		Ratio of tuberculous morbidity—vaccinated controls
			Vaccinated	Controls	
Indian children	1933-38	450-51	306	303	1 4.55
Adults—Nurses in general hospitals	1938-43	468-51	1 005	1 304	1 4.27
Adults—Employees in sanatoria	1935-43	406-51	479	274	1 5.03

normal. More, it seems unaffected by the three BCG strains successively used. At any rate, there is no startling drop in the immunizing power during that period of time.

On the other hand, in table 6 it will be noted that the protection ratio of 1.485 against tuberculous morbidity obtained by Ferguson, in Indians, from 1933 to 1938, with strain 450-S1, has not lessened in hospital nurses and sanatorium employees observed from 1938 to 1943 and vaccinated with strain 563-S1, which ratios are 1.427 and 1.53.

#### COMMENTS

That the production of lesions cannot be a criterion of virulence as

But the fact is that the virulence of the bacillus is not a function

chemical composition and parallel to its virulence (32). It seems that tubercle bacillus strains attenuated or dead, retain as a stable residuum of their original virulence a degree of toxicity proportional to that virulence.

RE THE ABOVE AND ALL MATTERS THEREIN COMING

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Prof Dr A SIEGENBEEK VAN HEUKELOM (Java). In Indonesia, I have been impressed by the differences in tuberculosis among Chinese and Indonesians. The former live mostly in the cities, under crowded, often dirty, surroundings. The latter are essentially rural, living in clean bamboo dwellings. Not only is tuberculosis more frequent among the Chinese, but also they are infected earlier in life.

Dr. A C UKIL (India): From the point of view of one working in eastern India, I find many unexplained factors in tuberculosis. For us, it is difficult to ascertain what proportion of cases represent the

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skin manifestations of tuberculosis, such as lupus vulgaris or other tuberculids.

Dr. LONG. I have had no experience with BCG in skin tuberculosis.  
Dr. FRAPPET. We have not made any observations on this point.

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# ABSTRACT OF DISCUSSION OF PAPERS BY DRS LONG AND FRAPPIER

From my experience with tuberculosis in the Scandinavian countries, I believe it to be true that in these countries one more frequently sees acute exudative, pneumonic tuberculosis following primary exposure than we observe it in the United States. Here, this eventuality seems to be more frequent among Negroes than among white patients.

must bear in mind that no vaccine can do more than can the spontaneous infection.

Dr MAHMOUD ABDEL AZIM (Egypt) I should like to comment upon the association of tuberculosis and bilharziosis. In patients with bilharziosis, tuberculosis is almost invariably mild. We not infrequently see post mortem evidence of fibrosis and healing, indication of a mild infection. Especially is this true of bilharzia patients with marked splenomegaly. Among these patients we have yet to see any significant



care. A 35 millimeter photofluorogram, a 4 by 10 inch stereophotorenogram, a roentgenogram on a 14- by 17 inch paper negative and a conventional 14 by 17 inch celluloid film were taken within a few minutes of one another, of the chest of each person participating in the study. The four sets of films were then interpreted independently by the five members forming the board. Prior to reviewing the films the board convened, reached agreements on nomenclature, and developed a code for classifying the films into distinct categories as uniformly as possible.

When the data were finally collected the results were hardly those that had been expected. A marked variation was found from one reader to another in the number of individuals called positive for tuberculosis. This variability was present not only when the readers interpreted the small 35 millimeter photofluorograms which might conceivably be difficult to read but also when they examined the 14 by 17 inch celluloid films. For example, in one group of 1,256 films of the 14 by 17 inch celluloid films, the number of films called positive for tuberculosis by 1 or more readers was 100.

The foregoing data were shocking and almost unbelievable to the several readers participating in the study, for in some cases as many as 20 percent of the films called positive for tuberculosis by 1 reader were called entirely negative by another. An attempt was made to attribute these differences to the varied background and experience of the 5 readers. However, this explanation remained tenable only for a brief time. After the readings on the 4 sets of films were completed, the 14 by 17 inch celluloid films were read by the 5 participants in the study.

The foregoing data were shocking and almost unbelievable to the several readers participating in the study, for in some cases as many as 20 percent of the films called positive for tuberculosis by 1 reader were called entirely negative by another. An attempt was made to attribute these differences to the varied background and experience of the 5 readers. However, this explanation remained tenable only for a brief time. After the readings on the 4 sets of films were completed, the 14 by 17 inch celluloid films were read by the 5 participants in the study.

films for tuberculosis is subject to two serious types of subjective error (1) *Interindividual errors* or the failure of one reader to be consistent with another in interpreting a film, and (2) *intraindividual errors* with himself in two independent readings. These errors are not of infrequent occurrence and are sufficiently large that they prevented Birkelo et al from making a thoroughly satisfactory assessment of the value of the four types of films for the diagnosis of tuberculosis.

## PROBLEMS IN THE XRAY DIAGNOSIS OF PULMONARY TUBERCULOSIS<sup>1</sup>

RUSSELL H MORGAN, M D, *Professor of Radiology, the Johns Hopkins Medical School, Baltimore, Md*

During the past several decades the application of roentgenologic methods to the diagnosis of pulmonary tuberculosis has become so well established that many physicians regard the finding of a poorly defined region of increased density in the upper lung field of a chest film as tantamount to the discovery of tubercle bacilli in the patient's sputum. Indeed, there are some physicians who are thoroughly convinced that they are able not only to detect the presence of a tuberculous process by means of a chest film alone but also to determine its activity. Unfortunately, the development of such confidence in roentgenologic procedures has not always been based on sound scientific ground. It is therefore not surprising that recently serious doubt was cast on the efficacy of the roentgenologic process as a detector of tuberculous pathology by a group of investigators working jointly in the Veterans' Administration and the United States Public Health

and frequently exhibit deficiencies apparently related to subjective defects of interpretation.

The results of the studies by Birkelo et al<sup>2</sup> received considerable

findings and to interpret them in terms that will be meaningful to the medical profession in general.

The investigation was originally begun in 1944 when the Veterans' Administration appointed a five man board of roentgenology to evaluate the diagnostic efficiencies of the various sizes of films, roentgenographic and photofluorographic, which were then available for the roentgenologic examination of the chest. In selecting the material on which to base its study, the board attempted to simulate as nearly as possible the conditions of mass survey work for which these media are ordinarily utilized. The entire populations of two Veterans Administration institutions were surveyed and included employees, ambulatory patients of a general hospital, and residents for domiciliary

<sup>1</sup> From the Department of Radiology the Johns Hopkins University Baltimore, Md.

# STREPTOMYCIN IN THE TREATMENT OF TUBERCULOSIS

WALSH McDERMOTT, M D, *Department of Medicine, Cornell University Medical School, New York, N Y*

It is only 4 years since Dr Feldman and Dr Hinshaw of the Mayo Clinic established the fact that streptomycin is effective in the treatment of tuberculosis.

by a number of groups and individual investigators all over the world. In this country, the principal studies have been conducted by the Veterans' Administration and by the Tuberculosis Study Section, of the National Institute of Health, working in conjunction with the American Trudeau Society. The results of these many investigations including our own, are all in essential agreement.

Today's report consists of a presentation of some of our own results observed on the Cornell New York Hospital Medical Service by Dr Carl Muschenheim and myself and our associates.

During the first 2½ years of the investigation, approximately 150 patients with various forms of tuberculous infection were treated with streptomycin. Four streptomycin regimens have been used successfully.

applied in which the streptomycin is administered twice weekly. The reasoning responsible for the changes in regimen will be presented subsequently.

1. — 1 — tuberculosis  
2, the dose  
meningitis  
daily three

For the purposes of evaluation, the cases have been grouped into four categories: (1) Tuberculous meningitis, (2) generalized hematogenous tuberculosis, i. e., miliary tuberculosis, (3) predominantly exudative pulmonary tuberculosis with or without cavitation, and (4) tuberculous disease of other organs.

Of the 150 patients with bacteriologically confirmed tuberculosis who have been treated, nine have died, one-tenth is dying, and hence in only two is there a possibility for cure within one year.

mental impairment.

*Miliary tuberculosis*—Thirteen patients with acute generalized hematogenous tuberculosis of the miliary type have been treated.

The large subjective error in the interpretation of chest roentgeno-

indicate that in 100 roentgenograms actually showing a moderate degree of tuberculosis, one may expect from even an experienced reader only fifty odd positive diagnoses.

The foregoing material must raise several questions (1) Can the published data by Birkelo et al be regarded as entirely reliable? (2) If so, how seriously are our roentgenologic concepts of tuberculosis invalidated? (3) How can these subjective errors be reduced?

In regard to the first question, there is little doubt that mathematically at least the published data are reliable. There is some doubt about the practical reliability of the data since no effort was made in

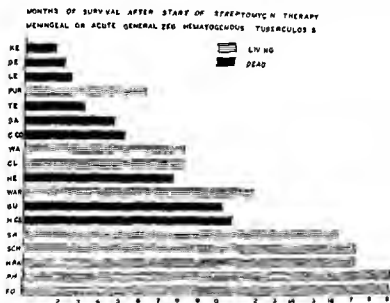
Yerushalmy has recently undertaken an investigation to determine this acumen, and the initial results seem to indicate that the subjective variation in interpretation from one reader to another or of one reader from one time of interpretation to another is even greater than that demonstrated in his earlier studies. Until more definitive studies are

the roentgenologic diagnosis of tuberculosis. No longer can we assume that when an individual is diagnosed as negative for tuberculous pathology he is actually free from the disease. Yerushalmy and

and (2) educational deficiencies in the training of the reader. Although few physicians are deliberately careless in their scrutiny of a roentgenogram, most have not organized their methods of examination to the point where adequate care can always be exercised. All too

examination. After 5 weeks of such remission, however, starting the one hundredth day of treatment, there was a return of the entire picture of acute miliary tuberculosis. Bacilli isolated from lymph and sputum at this time were highly resistant to streptomycin. Despite the continuation of treatment, this second bout of miliary tuberculosis was steadily progressive and terminated fatally 5 months after the original institution of therapy.

In a film obtained 3 months after the start of treatment, when the patient was in complete remission, it is impossible to detect any abnormalities. When relapse appeared, the films again presented characteristic appearance of miliary tuberculosis similar to the



treatment film. This same degree of roentgenologic clearing observed in all but 1 of the 13 patients with miliary tuberculosis; only 5 of the individuals, however, has the remission been sustained and maintained after the cessation of therapy.

(3) Representative examples of the type of result noted in patients with pulmonary tuberculosis show that the course of these infections under streptomycin followed one of three patterns. The first pattern consisted of a disappearance of symptoms accompanied by extensive roentgenologic clearing with cavity closure and reversal of infectiousness. As would be anticipated, this type of result was seen only predominantly exudative disease with relatively recent duration.

The change observed after 2 months of treatment of a 42-year-old white woman with a rapidly progressive exudative tuberculous pneumonia with cavitation is recorded in two films, taken 11 weeks apart. The clearing was accompanied by prompt closure of the cavity. D

### III BACTERIAL AND SPIROCHETAL DISEASES

(The term 'complete remission' is used to designate patients who are afebrile, asymptomatic, discharge no tubercle bacilli, and are free of all signs of active disease.)

of 4 to 6 months before fatal relapse. In figure 1, the duration of life after the start of streptomycin therapy is shown for 10 patients, chiefly adults, all of whom had either meningeal or miliary forms of tuberculosis. Each horizontal bar represents a patient's survival after therapy, and the total width indicates a 21-month period. The solid black bars represent patients who died, and the horizontally striped bars represent patients who are still alive. As may be seen, only the 5 patients at the top failed to survive 90 days, and several of the fatal cases survived 10 months or longer.

A representative example of a complete and sustained remission of miliary tuberculosis may be mentioned. The patient was an old white woman who presented the characteristic clinical, roentgenologic, and bacteriologic findings of acute miliary tuberculosis in July 1946. During the first 2 weeks after the start of

therapy, there was a complete remission of her infection until the present.

A result similar to this was observed in a total of 5 of 10 patients with acute miliary tuberculosis. The 2 causes of therapy were streptomycin and isoniazid.

disease.

An example of these phenomena was a 21-year-old man who was desperately ill with miliary tuberculosis and a considerable enlargement of lymph nodes when treatment was started in December 1945. Fever subsided and symptomatic improvement was in evidence by January 1946, but on the thirty-fifth day he developed signs of meningitis, which was treated intrathecally. At the end of that time (June 1, 1946) he was in complete remission.

was often followed by a relapse of the disease. In a small number of cases

tubercle bacilli despite the treatment

ancee of the phenomenon between the two diseases is that relapse does not necessarily follow the emergence of bacterial resistance in pulmonary tuberculosis. Thus, although streptomycin is undoubtedly an extremely powerful anti tuberculous drug, the period during which this action can be exerted in an individual case is sharply limited to 1, 2, or 3 months. Moreover, because of the chronic relapsing nature of tuberculosis and the persistence of drug resistance once it emerges, it is important to avoid the emergence if possible.

One way in which this can be done is by shortening the total duration of treatment. This is tantamount to foregoing

over, although the 42 day regimen seems to provide adequate anti microbial therapy for many pulmonary infections, it is inadequate in others because of its brevity. In order to reduce the phenomenon of bacterial resistance to a minimum by this approach, it would be necessary to limit the length of an individual course of therapy to less than 30 days. It is hoped, therefore, that investigations now in

known whether the therapeutic effect, but it is certainly close enough to it to warrant its usage in all but the most serious infections.

To summarize the problem of the types of tuberculosis in which

lerial therapy. At this time, it would seem that the only way to attain a satisfactory tuberculosis with or without months of powerful permanent remissions

appearance of the residual infiltration occurred soon after the cessation of therapy. Cultures of the sputum have been negative for the past year, and the patient is in a complete remission.

A similar type of response was observed in the majority of the patients with exudative disease of relatively recent duration.

The second type of response consists of a temporary period of improvement followed by relapse associated with drug resistant microorganisms. Only a minority of the predominantly exudative infections show this type of course, but it is frequently observed in the chronic fibro-cavernous forms of the disease.

An example of this course of temporary improvement followed by relapse under therapy was a 22 year old Negro woman with exudative tuberculosis of relatively recent duration. Defervescence appeared soon after the start of drug therapy, but the improvement persisted for only 3 weeks and was not accompanied by any roentgenologic clearing. The predominance of drug resistant tubercle bacilli was

lung, and ended in death 7 months after the first streptomycin therapy.

The third and most common type of course under streptomycin therapy is illustrated by a 39 year old Negro woman with confluent

course of antimicrobial therapy. Consequently, a three stage thoracoplasty was performed last July. The operation was immediately followed by cavity closure and reversal of infectiousness, and the remission has been maintained until the present time. The pleural thickening of the right lung is a consequence of a pleural effusion 3 years before the present illness.

This type of response, extensive clearing with cavity shrinkage which falls short of complete closure, is the most frequently observed course under streptomycin therapy. Moreover, it is believed that this type of case represents one of the greatest fields of usefulness in primary tuberculosis. In another case, which would have been distinctly in

stage to attempt collapse therapy of the confluent pneumonia. After temporary control of the progressive infection by drug therapy it was possible to correct the anatomic situation with a most satisfactory result.

In tuberculosis, as in other infections, when the administration of streptomycin is continued for a sufficient period, to patients with un-



In cases in which native resistance appears very poor, streptomycin may have little if any significant effect and the disease may quickly progress as soon as the administration of the drug is discontinued. We find support for the conception that the proper use of streptomycin often helps to bring active tuberculosis under control and provides

in order to consolidate the gain and avoid relapse

An intimate knowledge of the pathology and pathogenesis of tuberculosis is a prerequisite to the intelligent use of streptomycin. The extent and nature of all the lesions in a given case should be identified as accurately as possible. The patient's resistance should be judged and on the basis of this information a prognosis of the probable course and outcome should be made. On this basic appraisal, one may then determine whether to use streptomycin and, if so, whether its use should be immediate or whether it should be deferred for anticipated more urgent needs, also whether the course of treatment should be long or short, in the latter case with the possibility of effective retreatment later if necessary.

The dangers of the misuse of streptomycin in tuberculosis are greater than those of most antibiotics in other diseases. The most obvious of these dangers are

(a) Toxic damage from the drug without any lasting effect on

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ifying its

therapeutic effectiveness in later, more serious episodes

(c) Improper timing of the administration of streptomycin with

onse  
ions

themselves

(e) Failure to capitalize on the favorable effects of streptomycin by continuing rest treatment for a long period after its administration

Dr RICHARD A S CORR (Jamaica) We have treated only a few tuberculous patients with streptomycin. Those I have observed (mostly Negro) seem to do well for 3 to 4 weeks and then retrogress.

Dr McDERMOTT (in response to a question regarding the complications of streptomycin therapy) The toxic reactions to streptomycin therapy include anaphylactic reactions (fever, dermatitis) such as

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latter may

be minimized by using smaller doses for shorter periods of time but is almost universal when large doses are used over prolonged periods. Deafness is not a serious problem unless large doses of streptomycin are used

in a small but impressive minority of the individuals with acute military tuberculosis or meningitis. In the majority of pulmonary infections, however, particularly those of long standing, the lesions are of such a nature that extensive resolution and natural repair are not possible. In such situations, the administration of streptomycin results in only temporary improvement and the eventual emergence of drug resistant infections. From the experience to date, there is every reason to hope that many of these cases can be significantly aided by intelligently timed antimicrobial therapy used in conjunction with surgery.

#### ABSTRACT OF DISCUSSION

Dr J BURNS AMBERSON (United States), commentator. Our

some of our conceptions of the place of streptomycin in the treatment of tuberculosis, there are certain limitations within which this treatment should be confined in order to secure its maximum effect. These are

(a) Drug toxicity, which is minimized by limiting the dose of the drug to 1 gram a day or less and the course of administration to 6 weeks or less.

(b) Bacterial resistance to the drug which becomes rapidly manifest after 3 or 4 weeks of treatment. In our experience thus far, the proportion of pulmonary cases in which drug fastness developed is 30 percent or less if the course of treatment was limited to 6 weeks. Presumably shorter courses will reduce this percentage still further.

dergone necrosis and other destructive changes are less so. These changes are known to be related to the duration and severity of the lesions. Lesions in structures in which caseation is seldom extensive,

drug to bring about the abatement of symptoms, the control of inflammatory processes, and an acceleration of their resolution. Residual caseous lesions do not appear to be greatly influenced, and these can heal only slowly by fibrous organization with or without ulceration. Until then — — — — —

objectives such as the control of active lesions during thoracic surgery or the relief of distressing symptoms such as dysphagia due to laryngeal tuberculosis.

## SCHEDULES OF THERAPY

Since the discovery that penicillin was effective in the treatment of syphilis, numerous schedules varying from one another in dosage, interval between injections, and combination with other antisyphilitic therapy have been tried. With the exception of several very poor schedules and one unaccountably good schedule, the most striking finding of both evaluations is the similarity of results produced by the schedules employed to date, regardless of total amount of penicillin, dosage, or interval between injections.

Possibly one explanation for the similarity of results between high

therapeutic index

It is believed that the penicillin originally furnished in 1943 and early 1944 was predominantly penicillin G. In the latter part of 1944 and in 1945, there was a change in the relative fractions of

ferences in effectiveness among types of penicillin is demonstrated by the comparison of crystalline penicillin G with amorphous penicillin as supplied until mid 1946. This comparison indicates that crystalline penicillin G is considerably more effective than amorphous penicillin.

at 10 hours interval between schedules

y the same

when the identical total dosage is given in 3 hour intervals over 4 days, 8 days, or 15 days. This 3 hour interval between injections, with variations in the size of the dose and total duration of treatment, was most frequently used among schedules included in both evaluations. One schedule, however, which produced striking results consisted of 3,400,000 units of penicillin given in injections of 40,000 units every 2 hours. The failure rate at 12-15 months is about 4 percent, all other schedules utilizing amorphous penicillin exceed 10 percent at this same period of post treatment observation. Although in other schedules with a total dosage of 2,400,000 or 4,800,000 units the failure

rate was less than in the 3 hour schedules,

if crediting the 2 hour in

is affected by the 3,400,000

other schedules compared,

the results of this schedule are based on records from one institution

## Session 2 SYPHILIS, YAWS, PINTA AND RELAPSING FEVER

*Tuesday, May 11, 9 30 a m-12 m*  
*Departmental Auditorium, Main Hall*

### THE TREATMENT OF SYPHILIS WITH PENICILLIN

J R HELLER, Jr, *Chief Venereal Disease Division, United States*  
*Public Health Service, Washington, D C*

Since 1944 two cooperative evaluations of the effectiveness of peni

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State and locally sponsored rapid treatment centers. This paper

primary and secondary syphilis, results in the Venereal Disease Division analysis are based on previously untreated secondary syphilis

"retreatment" is dated at the actual time treatment is instituted, this procedure not only delays the rise in the retreatment curve but also changes the classification of failures. As a result, failures reported by

exclude reinfections from the failure or retreatment rates. In spite of these differences in the two evaluations, the findings to date, with but few exceptions, are in agreement.

For purposes of comparing treatment schedules, the 12 to 15 month period of post treatment observation has been selected, for it is felt that by this time the majority of infections relapses would have occurred and sufficient time would have elapsed for seroresistance to be determined. Use of this period also makes possible a presentation of some of the recent schedules of therapy which are of more general interest than schedules which have 2 to 3 years post treatment observation.

injection on Saturday and none on Sunday. Eighty seven percent completed treatment, 45 percent without missing a single injection. The third study was conducted by the State of Delaware in four clinics located in different cities. Clinic sessions were scheduled for 6, 7, 8, and 10 days only. Unlike the other two studies, if a patient missed a day he had no opportunity to complete treatment. Eighty seven percent attended all scheduled days.

### PENICILLIN REACTIONS

Penicillin in peanut oil and beeswax and aqueous penicillin occasionally produce allergic reactions, e.g., urticaria and angioneurotic edema. Pyrihenzamine and benadryl are useful in the treatment of such reactions, adrenalin being used as an emergency measure. A Herxheimer reaction of the systemic (fever) type occurs in about 50 percent of patients with early syphilis, and grossly visible focal (exacerbation of lesions) type in less than 10 percent of the early syphilis patients. Herxheimer reactions from penicillin cannot be entirely avoided even by starting treatment with small doses of the

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itis, and other react  
were severe. A severe  
among patients treated with aqueous penicillin alone and 31 among  
patients treated with penicillin in peanut oil and beeswax. Among  
a combination therapy of  
were 15 fatalities and a severe  
treated. Hemorrhagic en  
cephalitis was the principal  
it is questionable whether  
results accruing from the  
involved

### RECOMMENDED TREATMENT SCHEDULES

On the basis of information accumulated to date, the following schedules for early syphilis are recommended by the Syphilis Study Section, Research Grants and Fellowships Division and by the Venereal Disease Division.

and are composed predominantly of young white males with primary syphilis

The poorest results have been attained in very low dosage schedules (600,000 units or less) and in schedules of short duration (10 000 000 to 25,000,000 units administered by 1 day intravenous drip and from 600,000 to 2 400,000 units of penicillin in conjunction with 6 hours of

rate was more than 50 percent among patients with secondary syphilis treated with 10,000,000 to 25,000 000 units by 1 day intravenous drip

Eagle, Magnuson, and Fleischman have demonstrated with rabbits

deaths from treatment reported to the Public Health Service among cases treated in rapid treatment centers have been patients treated with schedules combining arsenoxide with penicillin

At the present time post treatment observation of 15 months is avail

amorphous penicillin given in the same period of time In both dosage groups, the cumulative failure rate of crystalline penicillin G is about 57 percent of the rate for amorphous penicillin

Amorphous penicillin in peanut oil and beeswax (P O B) is equally as effective as amorphous penicillin in aqueous solution Two P O B schedules employing 4 800,000 units in 8 days are available for comparison In one, 600,000 units were administered once a day, in the other, 300 000 units were given twice daily The failure rate for these 2 schedules is almost identical with the rate for the same amount of amorphous penicillin

economically feasible to provide daily clinic services at convenient hours. This has been demonstrated by three case holding studies In one, conducted by the State of Vermont in cooperation with private physicians, 99 percent of the patients completed treatment, 83 percent within the scheduled 8 days At the San Francisco City Clinic patients were scheduled for injections twice daily Monday through Friday, one

I mention this new possibility to show how tentative this report of

recent past

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- 1 4,800,000 units of crystalline penicillin G in aqueous solution, administered in injections of 50,000 units every 2 hours for 8 days, or
- 2 6,000,000 units of crystalline penicillin G in peanut oil and beeswax, 600,000 units every 24 hours for 10 days

On the basis of all the available evidence, it would seem safe to say that these schedules at the end of 15 months will have a retreatment rate of less than 10 percent.

#### FUTURE POSSIBILITIES IN PENICILLIN THERAPY

I should like now to turn for a moment to the possible future of syphilis therapy. As you know, penicillin in oil and beeswax, as developed by Dr. Romansky, represents a first successful step toward eliminating the need for hospitalizing patients receiving penicillin within a medical facility. In fact, for gonorrhea, P O B achieved the ultimate desideratum, completion of treatment and a very high percentage of cure with one injection of penicillin.

However, the treatment of syphilis, as well as of many other diseases, requires a more prolonged exposure to penicillin than can be achieved by the administration of one injection of P O B. It has, therefore, been the urgent quest of many investigators to find a medium in which penicillin can be administered, the physical or chemical properties of which would delay the absorption of the antibiotic for a sufficient time period so that completion of treatment could be achieved in one session.

considerable period, we cannot report with certainty to you what this period may be, but it would appear possible that it is about 3 to 5 days. Thus is, at the present time, only an estimate and cannot be used as a basis for recommended treatment schedules.

Recently a group of investigators has reported that procaine penicillin, with a particle size of less than 5 microns, in oil and aluminum monostearate is absorbed much more slowly than previously tested products. They find that more than 70 percent of patients tested re-

product show significant

It appears quite probable that procaine penicillin would give effective

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any single treatment cure will be successful for syphilis.



(2) We have used a regimen that involves the injection of 500 000 units of aqueous penicillin a day for 10 days, together with injections of bismuth subsalicylate twice a week. The preliminary results appear to be as good as those with POB or with 3 hourly injections of aqueous penicillin. We have also used penicillin in pectin gelatin suspension with favorable results and no allergic reactions.

Regarding pinta, our experience is unlike the results reported by Dr Varela. Our cases of pinta have responded well to penicillin. The serologic tests have behaved like those of patients with late latent syphilis, changing very little as a result of therapy. Nevertheless our experience is that penicillin is efficacious in cases of pinta.

Dr T B TURNER (United States) I should like to ask Dr Varela what proportion of his patients with early pinta have darkfield positive lesions, and how quickly these lesions become darkfield negative following penicillin therapy.

Dr VARELA (Mexico) All of our pinta patients have darkfield positive lesions. It is very easy to demonstrate large numbers of spirochetes in them. The organisms disappear rapidly from surface lesions following the administration of penicillin, but may again become positive.

Dr MOORE (United States) Dr Pardo Costello's contention is supported by work in experimental animals. In rabbits, penicillin administered in doses insufficient to give detectable blood levels will if given over long enough periods, cure the disease. The work of British investigators (especially Lourie of Liverpool) parallels Dr Pardo's findings. The question is not yet settled as to whether prolonged low concentrations or repeated peak concentrations of penicillin are more efficacious, but the former seems the more likely.

## PENICILLIN IN THE TREATMENT OF YAWS AND PINTA

Dr GERARDO VARELA, *Director, Instituto de Salubridad y Enfermedades Tropicales, Mexico, D F Mexico*

(Presented but not printed, as manuscript was not available)

### ABSTRACT OF DISCUSSION OF PAPERS BY DOCTORS HELLER AND VARELA

syphilis Penicillin is nearly 100 percent effective in preventing congenital syphilis. It seems to be more effective in the fetus than

Consider also penicillin in neurosyphilis. In all forms of syphilis of the central nervous system, from acute meningitis to dementia paralytica, the effects of penicillin upon cerebrospinal fluid abnormalities is more uniformly effective than in early syphilis. This is true despite the fact that penicillin does not penetrate neural tissues to any significant degree.

Although penicillin has solved many problems, it has raised others even more fundamental. We need more work on these problems.

Dr E. A. FRIEDMAN (United States) It has been my experience

West Africa and the Belgian Congo we have studied the usefulness of an organic arsenical given by mouth in the treatment of yaws.

preliminary results are entirely and show evidences of cases, and a period of ob-

servation of less than 3 months. Thus far, however, there have been no relapses, and we have observed a favorable effect upon serologic tests.

Dr V. PARDO-COSTELLO (Cuba) It is difficult to discuss Dr Heller's paper. The data upon which it is based are extensive indeed. I should like to comment on two points.

(1) The stress placed upon blood concentrations of penicillin. I question whether this is as important as in acute bacterial diseases. I believe tissue concentrations to be more important and suggest that these are probably of longer duration (as adjudged by urinary excretion) than is indicated by the blood level.

topigmentarias, o ligeramente acromiánicas, que evolucionan durante años

Durante el período secundario de la frambesia hay manifestaciones sistémicas que se traducen por fiebre, malestar general, dolores osteoarticulares, etc., que no hemos observado nunca en la pinta

El período tardío de la pinta y de la frambesia sólo tiene un punto de contacto: las queratodermias palmoplantares. Pero en la pinta tardía, por lo menos en México, solo se ve en un 5 al 10%, como máximo, de los enfermos y va acompañada constantemente de otras manifestaciones cutáneas que nunca se han señalado en la frambesia: melano dermitis difusa o en placas de la cara, cuello, antebrazos, piernas y de manchas acrómicas en las muñecas, codos, rodillas, tobillos, etc. En el 90 a 95% de los enfermos, solo existen las últimas manifestaciones cutáneas, sin queratodermia palmo plantar

En la frambesia tardía son frecuentes las osteoperiostitis deformantes que nunca se ven en la pinta, así como tampoco se ven en esta lesiones de tipo gomoso

Estas diferencias no pueden ser atribuidas a diferencias raciales o ecológicas, porque en países donde coexisten la pinta y la frambesia ambas treponemosis presentan, en individuos de la misma composición racial, los caracteres que hemos señalado

#### DIFERENCIA ENTRE LA SÍFILIS, LA FRAMBESIA Y LA PINTA EXPERIMENTALES EN LOS ANIMALES DE LABORATORIO

Hay diferencias patentes entre la sífilis y la pinta experimentales

nuestras manos, cuando las inoculaciones se realizan intradérmicamente en el escroto. En la pinta, a pesar de intentos repetidos, no hemos logrado producir orquitis, y sí solamente chancros escrotales que curan espontáneamente sin generalización de la infección

Lo mismo podemos decir en relación con la frambesia experimental en conejos

De todos es conocida la susceptibilidad de algunos monos inferiores, entre ellos *macacus rhesus*, a la sífilis y a la frambesia. En doce monos de esta especie el autor no ha logrado producir lesiones experimentales de pinta.

#### SUMARIO Y CONCLUSIONES

Los conocimientos adquiridos en los últimos diez años sobre la lesión inicial de la pinta y de la evolución de esta treponemosis, permiten encontrar diferencias fácilmente reconocibles entre la pinta o carate, por un lado, y la sífilis y el yaws, por otro

La lesión inicial de la pinta difiere, tanto desde el punto de vista morfológico como del de su evolución, del chancro sífilítico y de la lesión inicial de la frambesia trópica (yaws)

# LA PINTA O CARATE SU RELACIÓN CON LA SÍFILIS Y LA FRAMBESIA

Dr F LEON BLANCO, *Universidad de la Habana, Escuela de Medicina,  
Habana, Cuba*

Después de ballazgo de treponemas en un caso cubano de pinta realizado por Alfonso, Grau Triana, y León Blanco, y de los estudios clínico epidemiológicos y experimentales del autor en Cuba y México, la pinta ha quedado definitivamente incorporada al grupo de enferme

lizado, la pinta y la frambesia están confinadas a los tropicos

La patología general de estos tres treponemas presenta algunos puntos  
autores

causales, y a los puntos de contacto de su patología general creen que sífilis frambesia y pinta son una sola afección con manifestaciones clínico epidemiológicas distintas debido a la acción de factores raciales del huésped (el hombre), y a especiales condiciones ambientales que actúan sobre el huésped o sobre el parásito, o sobre ambos a la vez. Otros autores, cuya opinión compartimos, creen que sífilis, frambesia y pinta son tres enfermedades distintas, cada una de ellas producidas por un treponema específico *Treponema pallidum*, *Treponema pertenue* y *Treponema carateum*, respectivamente.

Esta divergencia de opiniones plantea un problema que puede enunciarse en estos términos: ¿Son la sífilis, la frambesia y la pinta tres síndromes clínico epidemiológicos distintos de una única entidad nosológica, o por el contrario, son en sí mismas tres enfermedades distintas? Llevado al terreno puramente biológico podríamos formular más claramente el problema en estos términos: ¿*Treponema pallidum*, *Treponema pertenue* y *Treponema carateum* son tres especies específicas del género *Treponema*, o sólo nombres distintos de una misma especie?

La respuesta a este problema principal está subordinada a las que puedan darse a estas dos preguntas:

(1a) ¿Hay diferencias fácilmente demostrables entre la sífilis, la frambesia y la pinta humanas, y entre las lesiones que se obtienen en los animales de experimentación, según que el inóculo haya sido tomado de un caso de sífilis, de frambesia y de pinta?

(2a) Si existen estas diferencias ¿son debidas a diferencias en el huésped y a los factores ambientales que rodean al huésped, o se deben a diferencias biológicas inherentes al microorganismo infectante?

and reasoning backward to assume that each syndrome must be due to a different parasite. This is creation of species by fiat. No taxon

the host and the physical environment, and the results of these interactions are the clinical syndromes. Take the parasite by itself and it is always *T. pallidum*. Put the parasite in the rural population of Haiti and the resultant disease is Haitian yaws. Combine the parasite with the urban population of Kingston, Jamaica, and the result is venereal syphilis. Turn the parasite loose in Guam and there is a preponderance of gangosa yaws, turn it loose among the aborigines of Australia and the result is boomerang tibiae, put the parasite to work in Central America and there is a preponderance of depigmentation.

The final effects of the parasite upon man have, it is true, an infinite variety, but all these are variations on a central theme, the basic pathology of treponematoses. Prolonged study of this pathology, whether in yaws, pinta, or syphilis, only makes more clear that the pattern is essentially one, and that the differences are quantitative, not qualitative, of degree and not of essence.

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pendent disease with a specific parasite stumbles on the hard fact that there is "pinta" to some degree in Guam and Arabia and Central Africa, indeed everywhere that there is treponematoses.

Pinta is a useful descriptive term for a syndrome of treponematoses. Any decision to give this clinical syndrome the dignity of a separate

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room under this tent not only for those who believe that *Treponema* has only one species but also for those who believe there are two, or three, or more, just so we can all agree it is one disease.

Dr G. C. SHATTUCK (United States). For a long time I was convinced that syphilis and yaws are different diseases. The clinical picture in the early stages differ, but in the tertiary stage, the two are indistinguishable. As to pinta, I find this condition difficult to classify. Perhaps we should reinvestigate vitiligo. Now I find myself in general agreement with Dr. Hudson's thesis of unification. The three conditions probably are different manifestations of one disease.

### III BACTERIAL AND SPIROCHETAL DISEASES

Aunque hay alguna semejanza entre ciertas manifestaciones período secundario de la pinta, sífilis y yaws, consideradas aismente, no es menos cierto que en conjunto poseen características clínicas y evolutivas propias, que permiten diferenciarlas. Lo mismo puede decirse de las manifestaciones tardías de estas tres treponemias. Estas diferencias no pueden explicarse sobre la base de la diferencia de razas, de la distinta edad en el momento de adquirir la infección, la puerta de entrada del agente infeccioso, ni del estado económico social de los individuos afectados.

Ciertos factores ecológicos y el modo de vivir parecen desempeñar algún papel en la determinación de la distribución geográfica de la pinta y del yaws, pero no hay hechos que prueben que tales factores modifiquen el curso de la pinta, de la sífilis y del yaws en los individuos que viven bajo la influencia de tales factores.

Aunque aun faltan por realizar estudios extensos y más completos sobre la pinta experimental en el conejo, los hechos hasta ahora adquiridos parecen demostrar que aunque el chanero escrotoal pinto, sifilítico y frambesico son similares morfológicamente, hay una marcada diferencia de susceptibilidad del conejo a desarrollar chanero pinto, sifilítico y frambesico que hay diferencias apreciables por un lado, y chanero sifilítico y frambesico por otro. Como conclusión se establece que hay diferencias se deban a que *Treponema carateum*, *Treponema pertenue* y *Treponema pallidum* poseen distintas propiedades patogénicas, siendo por tanto tres especies distintas.

#### Abstract of Discussion

Dr. Ellis Herndon Hodson (United States), commentator. Señor León Blanco believes that because syphilis and pinta present different clinical pictures they must be caused by parasites of different species. Exactly the same argument has long been employed to justify applying the name *T. pertenue* to the treponeme associated with yaws, but the parasites of the three conditions are indistinguishable. If three spirochetes were put into the hands of a scientist and he were told that one was from a case of syphilis, one from a case of yaws, and the third from a case of pinta, he could not certainly determine which was which by any known test, visual, chemical, or biological. The philosophy underlying the assignment of specific names to indistinguishable parasites on the basis of their association with certain clinical syndromes, simply because these syndromes are different from each other, has the weight of conventional acceptance. It is high time for this philosophy to be challenged. When the etiological agents of parasitic diseases originally came to be identified, each parasite was given credit for its resultant disease, no matter how protean its manifestations. Only in spirochetal infection have we gone on naming syndromes and symptoms as diseases,

Dr. JOACHIM MOTTA (Brazil): These are different diseases despite

from the other. Pinta is rare in Brazil. We had no experience with it until the disease was introduced into our country during an international exposition.

Dr. A. L. BRUCE ROSSI (Venezuela): We too have seen syphilis and yaws exist side by side. We have seen, moreover, syphilitic patients develop pinta and patients with carate develop yaws. We have tried to differentiate these conditions serologically, and have had some success with tests which involve ether extraction of the serum. Thus we must accept the concept that these are not one but different diseases.

Prof C M HASSELMAN (Germany) I had hoped to have heard the last of the unitarian view of the treponematoses. The Latin American workers should be congratulated for their work on pinta. I have had little experience with this condition, but in Cuba I was impressed with the differences between it and yaws. The pigment is different, and it is far easier to demonstrate treponemes. Other differences include the differences in incubation period and the fact that in pinta there is no disruption of the epithelial surface. The last I consider a highly distinctive feature. In Dr Varela's observation that penicillin is none too effective in pinta, I see another indication that this condition is a separate and distinct entity.

There are, according to the observations of the larger group we know as the treponematoses.

Dr T B TURNER (United States) Everyone agrees that syphilis and yaws and pinta exhibit similarities as well as differences. They

spirochetes of syphilis and yaws produce distinctive disease pictures in rabbits over many generations. Another member of this group is venereal spirochetosis of rabbits (*T. cuniculi*). Would Dr Hudson simply refer to this as treponematoses?

there are, according to differences be microscope

retain any doubt but that they are different. I agree with Dr Turner that it is helpful to consider these as separate entities. Only when we can culture the organisms on artificial media, shall we be able to answer the question of whether these are different species or one species with three varieties.



Voici les caractères du liquide céphalo rachidien recueillis dans une série de cas—la lymphocytose étant prédominante (80 à 90%—contrôlés par inoculation positive du L C R a la souris)

	Cytologie	Albumine	Benjoin Colloidal
1 (Avant traitement)	85	0.85	00000 222 000 000
2 (Après traitement)	8	35	
3	160	28	
4	264	85	
5 Avant traitement	208	80	
6	( )	80	600 000 222 1 000 000
7	32	40	
8	102	65	0 III 000 222 1 00 000
9	110	45	
10	78	35	
11	82	40	
12	15	35	
12	212	85	
13	137	35	
14	( )	1 gr	211 000 000 0000
15	500	80	
16	340	85	
17	70	22	
18	10	22	
19	38	40	1222 000 000 000 00
20	98	45	
21	180	71	
	33	70	0 222 000000 000 000

Incomptables.

L'hypercytose est donc essentiellement variable allant de 10 à 15 lymphocytes jusqu'à plusieurs milliers (incomptables) Par contre le chiffre le plus élevé de l'albuminorachie (cas 13) ne dépasse pas 1 gr

La réaction de benjoin colloidal est souvent positive dans les premières tubes

Nous n'avons jamais observé de spirochetes dans le culot de centrifugation du liquide céphalo rachidien à l'examen direct

Par contre, l'inoculation du L C R a la souris donne des résultats aussi fidèles et aussi

C'est sur ce procédé  
liquide céphalo rachidien  
méningée de la fièvre récurrente et baser le critérium de la guérison

*Paralysies périphériques*—Les nerfs les plus fréquemment atteints

droite de type périphérique deux mois après le début de la fièvre récurrente chez un malade ayant présenté antérieurement une atteinte méningée sévère, alors qu'il paraissait cliniquement guéri et apyretique depuis vingt jours

# NOTE SUR LES FORMES NERVEUSES DE LA FIÈVRE RÉCURRENTE—FIÈVRE RÉCURRENTE À TIQUES EN AFRIQUE OCCIDENTALE FRANÇAISE

MÉDECIN LIEUTENANT COLONEL C F J BERGERET et MÉDECIN COMMANDANT A RAOULT, *Dakar, Afrique Occidentale Française*

Après les premiers travaux d'André et Marcel Léger (1917-18), ceux de Mathis (1926) et de Durioux (1931) firent connaître l'existence de la fièvre récurrente à *S. duttoni* dans la péninsule du Cap Vert et mirent en évidence les principaux chaînons épidémiologiques de cette maladie dont les cas annuels se sont accrus entre les années 1942 et 1946 (pour la seule ville de Dakar, de 26 à 85 cas hospitalisés)

L'affinité du spirochète de Dutton pour les espaces sous-rachnoïdiens ne tarda pas à frapper les médecins (Vialatte, Advier, Alain, Riou) et cette impression s'est confirmée par la suite

Le neurotropisme du spirochète de Dutton est un fait acquis et qui

réservoir de virus où ce dernier se conserve très longtemps

Les formes nerveuses de la fièvre récurrente donnent à la maladie un cachet particulier. Il faut distinguer

- (1) les formes méningées pures;
- (2) les paralysies périphériques,
- (3) les formes médullaires; et
- (4) les formes méningo-encephalitiques

*Formes méningées pures*—La fréquence de l'atteinte méningée prédominante, appréciée sur 57 malades hospitalisés, est de 48% soit, en gros, un malade sur deux

C'est, d'après les cas moyens, vers la fin de la 3ème semaine de la maladie que l'on commence à trouver une réaction normale du liquide céphalo-rachidien

La céphalée qui fait partie des maîtres symptômes du début de la récurrente, revêtant souvent une intensité extrême, est vraisemblablement liée à un processus de méningo-vascularite qui ne s'objective que plus tard dans le L. C. R.

On ne peut parler de "formes méningées" que dans les cas où le tableau clinique est celui d'une méningite aiguë: céphalée atroce, vomissements, constipation, photophobie, signes de Kernig, hyperréflex

Mme N'D., femme Dioula de 32 ans, est hospitalisée à l'Hôpital Central Indigène de Dakar pour une paraplégie spasmodique qui s'est installée une quinzaine de jours avant, à la suite d'une période prodromique faite de sensations de fourmillements dans les jambes, de douleurs radiculaires dans le domaine du nerf sciatique et d'hyperesthésie cutanée. L'impotence a été totale au début, clouant la malade au lit. Puis, il y eut une légère amélioration et elle a pu, en s'appuyant sur son père et sa mère, faire quelques pas pour attendre la voiture ambulance qui l'a conduite à l'hôpital.

Les mouvements actifs sont très limités, la malade fléchit très légèrement les genoux sans pouvoir décoller les talons du lit. La force segmentaire est presque nulle des deux côtés.

Réflexes ostéotendineux vifs, polycinétiques—Babinski en extension—Pas de clonus de la rotule, ni de trépidation épileptoïde du pied.

Hyperesthésie cutanée—Persistance des douleurs radiculaires; douleurs à la pression des masses musculaires—Pas de troubles sphinctériens.

L'examen des autres appareils ne montre rien de particulier. L'ir-

... cette femme est atteinte depuis un a  
nt trois o  
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céphalées, de vertiges, de bourdonnements d'oreilles, d'éblouissement au soleil.

Un traitement par la quinacrine et la quinine n'a pas eu raison de cette fièvre. Par ailleurs, la rate n'a pas augmenté de volume.

La ponction lombaire retire un liquide clair, non hypertendu, dont l'analyse donne les résultats suivants:

Cytologie: 43 éléments: chlorure: 7 gr. 0/00

Sels et pigments; néant.

Sucre: néant.

Chlorure: 9 gr. 40.

Un traitement est alors institué (try- rapidement une disparition totale de l'écoulement quitte l'hôpital marchant normalement à son arrivée. Une deuxième inoculation pratiquée 15 jours avant la sortie est suivie d'observation de l'animal.

Formes méningo-encéphaliques  
Gonnet et Gallais, "Médecine Tropicale"

Evolution très rapide vers la guérison sans séquelles en dix jours le malade n ayant reçu que deux injections de 1 gr 50 de tryparsamide pendant cet intervalle

*Observation 35* M M malade depuis le début de Juin 1945 récurrente à forme méningée le 2 Juillet Reçoit six injections d ace

la paralysie faciale très rapide en une dizaine de jours Traitement tryparsamide quatre injections de 1 gramme a 4 jours d intervalle

*Observation 31* Mme M, paresie faciale inférieure survenue trois semaines apres le début d une recurrenre classique—legere reaction méningée (25 éléments, 0,25 d albumine au moment de la paralysie faciale) Disparition totale spontanée en 36 heures

*Observation 34* Mme HA paralysie faciale droite totale périphérique avec début d ulcère trophique de la cornée coïncidant avec l'apparition des signes méningés survenant un mois apres le début de la maladie. Ponction lombaire 137 éléments 0 55 d albumine.

*Obser*  
incidan  
d en no

Après le facial les atteintes vestibulaires semblent relativement fréquentes Il n'est pas rare qu'au cours de l'évolution fébrile et

Signalons qu'on rencontre soit isolée soit en association avec la paralysie du VII<sup>e</sup>, une atteinte du trijumeau se traduisant soit par des névralgies intenses soit par une hyperesthésie voire même une anesthésie de la face et de la cornée

*Formes médullaires* —Déjà signalée par Vialatte puis par Advier Alam et Rou les formes médullaires sont intéressantes à connaître en raison de la complexité étiologique que revêtent souvent les paraplégies en milieu indigène en particulier

Voici un cas particulièrement net dont nous citons l'observation in extenso

L'impression générale est que la tryparsamide, employée seule, a bien une action très efficace et très rapide sur les phénomènes méninges, l'atteinte encéphalitique et les névrites périphériques

Par contre, l'apyrexie n'est pas obtenue d'une façon aussi brutale et aussi franche qu'avec l'acétylarsan

La stérilisation est moins sûre qu'avec l'acétylarsan et l'Orsanine.

Il faut noter cependant que nous avons surtout employé la tryparsamide dans les formes d'allures très sévères et que le traitement institué en pleine hyperthermie n'a donné lieu à aucun accident, bien au contraire

Enfin, si la guérison de certains malades a paru longue à obtenir, elle a pu néanmoins être totale comme l'indiquent les contrôles du liquide céphalo rachidien

Il semble que l'association moranyl + tryparsamide (moranyl 0 gr 50 et tryparsamide 1 gr 50 pour un adulte) mérite d'être reprise. Elle n'a été utilisée par nous qu'exceptionnellement

En bref, pour 11 cas attaqués à la tryparsamide, le bilan s'établit ainsi

5 guérisons rapides et complètes de formes graves (dont une méningo encéphalite),

4 guérisons apparentes suivies de rechutes fébriles, dans un cas, la maladie cède à des doses accrues et prolongées de tryparsamide, dans un autre à l'association tryparsamide moranyl, dans le troisième au stovarsol, dans le quatrième enfin au sulfarsénol,

1 guérison rapide dans un cas traité au début,

1 échec dû vraisemblablement à un traitement insuffisant

#### ACÉTYLARSAN

Parmi les cas de récurrente traités à l'Hôpital Principal en 1944-45, on relève 17

Pour 6 d'

ratiquée  
ut signé  
on complète  
lement, mais

parfois plus lente à venir

Parmi les 11 autres, trois présentaient un L. C. R. normal et huit une atteinte méningée manifeste précédant ou non la mise en traitement

En résumé, les 17 cas dont il est fait état se répartissent ainsi

12 succès à peu près tous incontestables

5 échecs, assez rapidement réparés grâce à la tryparsamide

L'un de nous a enregistré un syndrome cérébelleux et une forme hypersomnique ces deux cas ont été traités avec succès par la tryparsamide.

On connaît par ailleurs des formes psychiâtriques manie aiguë, confusion mentale, délires

L'existence de ces formes traduisent une atteinte parenchymateuse de l'encéphale permettant de parler de méningoencéphalite au cours de la fièvre récurrente

L'anatomie pathologique expérimentale, à défaut de faits précis observés chez l'homme, montre d'ailleurs (Levaditi) qu'il existe chez le lapin et chez le singe, des signes évidents d'encéphalite, manchons périvasculaires ou prédominant les plasmocytes et infiltrations lympho-monocytaires intraparenchymateuse sous formes de foyers discrets

#### EVOLUTION

Les formes nerveuses de la fièvre récurrente sont souvent tenaces et susceptibles de rechûtes

Toutefois, la maladie demeure quo ad vitam d'un pronostic bénin et la mortalité est insignifiante, mais l'intensité de la céphalée, la longue durée des paralysies faciales lorsqu'elles ne sont pas traitées, la gravité de certaines complications oculaires (qui, étant d'apport sanguin n'ont pas été envisagées ici) constituent des infirmités passagères fort désagréables

#### TRAITEMENT

Le traitement auquel nous avons recours avec le maximum de succès utilise l'acétylarsan et la tryparsamide.

#### TRYPARSAMIDE

La tryparsamide est utilisée depuis de nombreuses années par les médecins coloniaux dans le traitement de la fièvre récurrente à tiques. Comme pour la trypanosomiose, le gros avantage qu'offre ce médicament est d'agir certainement sur les complications encéphalo-méningées.

Nous l'utilisons habituellement par voie intra veineuse à la dose de

novocaine à 1% par voie intraveineuse

Des observations recueillies par Grall, Garcin et l'un de nous, on

dants dans le sang  
réactivation qui se  
pénicilline où l'on  
coup de fouet avant de s'effacer ultérieurement.

Depuis ces premières tentatives, nous avons utilisé des doses plus importantes: 2 500 000 à 3 000 000 Unités oxford en une semaine, les résultats ont été beaucoup plus satisfaisants

Les travaux expérimentaux de Levaditi ont montré, tout récemment, que, pour obtenir une stérilisation de la souris après une certaine durée d'évolution de l'infection par le spirochète, il fallait employer des doses très élevées de pénicilline

Il semble qu'il en soit même pour la maladie de l'homme

L'inconvénient de ce médicament réside, nous semble-t-il, dans sa faible diffusibilité à travers la barrière méningée. Il y aurait lieu dans le cas des formes nerveuses de la fièvre récurrente, de l'injecter dans le canal rachidien; nous n'avons pas eu l'occasion de l'utiliser par cette voie jusqu'à présent.

Une observation de Lebon et Choussat (Algérie Médicale, Septembre-décembre 1945) nous paraît intéressante à ce sujet. Dans cette observation, en dépit d'un tableau clinique très alarmant de méningomyélite aiguë et après l'échec de la thérapeutique arsenicale (sulfarsénol) la guérison fut obtenue par la pénicilline injectée à la fin par la voie intra-musculaire et intra rachidienne à la dose de 500 000 unités. Il s'agissait d'une fièvre récurrente cosmopolite.

#### ABSTRACT OF DISCUSSION

Dr MALCOLM H SOULE (United States), commentator Lieutenant Colonel Bergeret has given an excellent presentation of a type of relapsing fever which we rarely encounter in this hemisphere. All students of the disease recognize its protean manifestations and the importance of demonstrating the spirochete in order to confirm the diagnosis suggested on the basis of the clinical findings. The carrier state and premunition are important aspects of the tick-borne malady.

The relapse phenomenon remains one of the outstanding enigmas of this infection. It has been explained in terms of an inherent capacity

relapse phenomenon  
cells with the aid of  
ve been removed at

Il est à noter que, parmi les échecs, certains peuvent être attribués à une conduite defectueuse du traitement. Il semble aussi qu'il soit

En effet, des chocs sérieux, voire graves peuvent se produire lorsque des injections sont faites en pleine recrudescence fébrile

De ces observations, malgré l'insuffisance des contrôles sanguins et du liquide céphalo rachidien, il ressort que l'acetylarsan, arsenical très

Pour clore ce chapitre consacré à la très classique thérapeutique arsenicale, voici schématiquement comment peut être conduit le traitement de la récurrente dakaroise

1<sup>er</sup> Traitement — A la phase d'attaque des la confirma-  
préférence chez l'européen—on  
injection tous les trois jours six  
injections au total, puis, tryparsamide—2 centigrammes par kilo—  
une injection tous les quatre jours en surveillant l'apparition des  
phénomènes méningés

A la phase méningée continuer la tryparsamide si elle a été com-  
mencée jusqu'à 20 injections suivant le même rythme. Commencer  
d'emblée par la tryparsamide si on observe le malade à ce stade

2<sup>e</sup> Traitement symptomatique — La ponction lombaire est à la fois  
un geste de diagnostic et un excellent traitement de l'hypertension  
liquorienne. Elle est d'ailleurs généralement bien acceptée par les  
malades, lors de la période où les céphalées sont au premier plan de  
la symptomatologie

Par contre, elle a beaucoup moins de succès lorsqu'elle apparaît  
comme un moyen de contrôle au moment de la convalescence

Le sérum hypertonique glucose intraveineux apporte un réel soulage-  
ment, malheureusement assez bref

Les calmants habituels n'ont qu'une action très faible sur les algies.

3<sup>e</sup> Traitement des neurites périphériques — La vitamine B1 et les  
moyens physiothérapiques habituels ne sont qu'un appoint secondaire  
au traitement étiologique par la tryparsamide

#### PENICILLINE

A la c . . . . .



## Session 3 PLAGUE

Tuesday, May 11th—2 00 to 4 30 p m  
Department of Commerce Auditorium

### EPIDEMIOLOGIA DE LA PESTE EN LAS AMERICAS

ATILIO MACCHIAVELLO, M D, Dr P H  
Pan American Sanitary Bureau, Lima, Peru

#### INTRODUCCIÓN

y por la unidad bacteriológica del agente infeccioso, la *Pasteurella pestis*

Con excepción de los Estados Unidos—en que se hace precozmente selvática—la peste en América progresa en 4 etapas. De 1899 a 1910, invade los puertos, como extensión de la pandemia asiática. Desde 1908 a 1920, el tráfico ferroviario y el comercio la introducen primero a la

por  
sión

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infecto...  
cuyo estudio fundamenta este trabajo

#### PESTE EN LOS PUERTOS

La peste portuaria carece de fisionomía continental propia. Introducida por el tráfico marítimo internacional y diseminada por el cabotaje costero, quedó influida por los factores primarios conocidos a saber, *Past pestis*, *Ratt rattus rattus* y *rattus alexandrinus*, *X cheopis*, *X brasiliensis* y factores sociales—como latitud, clima, tamaño y condiciones sanitarias—son la base de las diferencias observadas en los procesos

evaporación y lluvias y  
influencia sobre el vector

limitó la peste entre los paralelos 45° N. Norte y Sur, permitió el aumento de la *X cheopis* hacia el ecuador y el incremento inverso de la

intervals of twelve hours from purposefully infected rats, the spirochetes separated and photographed. In some animals four, or even more, relapses will occur. No differences were noted in the morphology of the organisms during the relapses at variance from the findings during the primary infection. One important observation has been

lapse phenomenon is worthy of additional investigation

## PESTE URBANA Y URBANO RURAL DEL HINTERLAND

La peste se internó en el continente vehiculada por ratas o pulgas utilizando el tráfico ferroviario, las rodovías, el comercio fluvial el transporte a lomo de bestia, el comercio de animales infectados, especialmente cobayos, el desplazamiento humano y el desplazamiento por contigüidad de roedores. El radio de dispersión de las pulgas infectadas, es, con mucho, más amplio que el de las ratas. Muchos puntos del *hinterland* se han mostrado refractarios a la peste o la aceptan bajo condiciones restringidas, sea por la ausencia de hospederos murinos, o de vectores, o por razones climáticas. Establecida la peste en forma enzoótica, su evolución semeja la de los puertos.

Cuando la ciudad infectada mantiene activo comercio con la zona rural circundante, la peste repercute en ella, formando focos múltiples, transitorios, que desaparecen junto con la eliminación de la peste urbana, excepto cuando el fenómeno rural depende de complejos movimientos de la población murina de ciudad a campo y vice versa, como acontece en las ciudades circundadas de cultivos de cereales.

Entre los mecanismos de extinción de la peste del *hinterland* en la zona andina, por arriba de los 3000 m, mereco tal vez considerarse la no existencia de *X. cheopis*, reemplazada como vector por la *N. longidens*, poco propicia a la perpetuación de la infección.

## PESTE RURAL

La peste rural en América corresponde a dos condiciones diferentes, (1) la peste rural campestre, o peste rural pura, en que interviene exclusivamente el *Rattus rattus*, con sus tres subespecies, *rattus alexandrinus* y *frugivorus* y en que el clima favorece la mantención del

3 a 5 años, tiempo promedio de reposición de las comunidades murinas devastadas por la peste, o por otras epizootias de distinta etiología.

La perpetuación de esta forma de peste rural se hace, o por una migración circulante progresiva y re épocas de cosechas, o por francas y extensas migraciones murinas en masa a través de centenares de kilómetros movimientos condicionados muy posiblemente, por la busca de la Vitamina E de los cereales y en los cuales la peste es un epifenómeno, ya que pueden observarse independientemente de ella.

En la Sierra del Ecuador y del Perú, la domesticidad del *Cavia aperea*, o cuy, complica este tipo de peste rural, sirviendo de reforzador de la misma, recolectando las pulgas libres infectadas, y dejándolas a

*N. fasciatus* por fuera de los trópicos, reguló las relaciones cuantitativas de ambas especies con hospederos y nidios, la distribución relativa por especies y sexo, la actividad alimenticia, la capacidad vectora, la longevidad y sobrevivencia en diversas condiciones ambientales y además determinó—conjuntamente con los cambios en número y en inmunidad de la población murina—el ciclo estacional de la peste.

A su vez, las condiciones sociales, urbanísticas, sanitarias y económicas de los puertos, influyeron sobre el volumen y distribución de la población murina en conglomerados confluentes o focales, abiertos o cerrados, determinando las facilidades de anidamiento, procreación, alimentación y proximidad al hombre.

Estas generalidades hacen comprender la dinámica de la peste portuaria. Los factores favorables auspiciaron la epizootia murina inicial violenta, aun en puertos que, como Seattle y Valparaíso, que dan localizados en los extremos de la banda pestosa. Cambiada la importancia de los factores iniciales, por la propia epizootia y por el clima, la peste, o desapareció espontáneamente, o se perpetuó en enzootias, con recrudescencias estacionales. Las reinfecciones fueron frecuentes, no siempre provenientes de puertos vecinos, como lo prueban las investigaciones de Long y Mostajo y las nuestras sobre las reinfecciones de los puertos del Pacífico Sur por pulgas infectadas provenientes de la India en fardos de sacos de yute.

La mantención interestacional de la peste se hace en el vector mismo, o en la continuación lenta y subterránea de epizootias murinas. Como en el primer caso, la virulencia de la *Pasteurella pestis* decrece en las pulgas en ayunas, y como la sobrevivencia del vector es incompatible con el bloqueo y la ausencia del bloqueo incompatible con la transmisión de la infección, queda por explicar el mecanismo por el cual la pulga infectada que actúa como reservorio se hace infectante. Sospechamos que la alimentación en animales susceptibles facilita el bloqueo pulido y la reposición de la virulencia de la *pasteurella*. En las ratas, la infección inaparente o mitigada, con bacteremia pestosa transitoria, pero demostrable, puede exaltarse (espontánea y experimentalmente) en roedores hembras preñadas, produciendo peste aguda septicémica, lo que explica la reactivación *in situ* de la virulencia de la *Past. pestis*.

A su vez, la extinción de la peste portuaria ha sido explicada por

lentas con agotamiento en plena estación pestosa del combustible epizootico, (5) intensa destrucción de vectores en estaciones desfavorables prolongadas, (6) desarrollo de refractariedad murina a la peste, o posiblemente, acumulación porcentual elevada de inmunes por infecciones atenuadas en zonas largamente enzooticas, etc.

## (3) Falta de correlación entre la frecuencia de las epizootias y las

ción

(5) Principal reservorio de la infección es la pulga, pudiendo jugar rol de dispersadores los cricétidos y aves de rapina y, de mantenedores, animales no hibernantes

(6) En las ardillas, posible infección latente sujeta a reactivaciones

(7) Casos humanos raros, infección por cualquier medio de contacto con roedores o sus pulgas, tipos clínicos comunes

(8) Posible retrocesión, o reversión de la peste selvática a roedores domésticos, debido al intercambio de pulgas

En Sud América, existen 3 focos conocidos de peste selvática: el de la Pampa Argentina, el que describimos en 1946 en la frontera del Perú con Ecuador y el de la región andina montañosa de Huancabamba que aquí relatamos por primera vez

En la Argentina, la peste selvática se extiende desde Jujuy a Rio Negro y La Pampa, abarcando variantes geográficas y fisiográficas que van del semi desierto al bosque y de la montaña a la zona agrícola plana, con climas variables, al igual que las lluvias. El hospedador y reservorio primitivo de la infección son los cuises, de los géneros *Cavia*, *Galea* y *Microcavia*, y posiblemente también el cricétido *Graomys griseoflavus* y especies afines, todas arborícolas. Los cuises viven en colonias, cavan madrigueras o tuneles entre las hierbas altas tienen poca afinidad por el hombre, procrean hacia la primavera y sufren conjuntamente tremendas epizootias pestosas que hacen desaparecer las colonias hasta que nuevamente se reponen, cada 3 a 5 años dando a la peste el carácter cíclico. Lagomorfos, lagostomus y cricétidos, pueden participar en las epizootias. Los índices pulidos por especie de roedores son variables, existiendo gran intercambio. A las 53 especies pulidas descritas por Del Ponte y Rüssel, representando 19 géneros, Jordan agrega 13 especies nuevas, de las cuales solo *Delastichus talis* y *Polygenis platensis cisandinus*, han sido sospechadas como vectores. Resumiendo los extensos trabajos de De la Barrera, Savino, y Alvarado, puede decirse que la peste selvática argentina, es esencialmente peste de *Cavia* y *Graomys*, con participación accidental y secundaria de otros roedores, en parte peridomésticos. Las infecciones humanas son escasas.

La peste selvática de la frontera peruano ecuatoriana, se extiende alrededor del paralelo 4° S y del meridiano 80° 2' 0 Gr, ocupando mesetas y laderas de cerros bajos, con matorrales y bosques sin caracteres de jungla, de clima seco durante 7 meses del año, y lluvioso el resto. La peste primitiva de la única especie de ardilla arborea *Sciurus stramineus neboru*, se ha extendido especialmente a tres especies de cricétidos: el *Oryzomys xanthaeolus xanthaeolus*, el *Rhipidomys equatoris*, y el *Akodon mollis*. Desde que el hombre ha aprove

su muerte nuevamente libres y en aptitud de alcanzar más fácilmente al hombre

(2) El segundo tipo de peste rural, lo llamamos *rural agreste*, porque en él, a la epizootia del *Rattus*, se suma, como epifenomeno temporal y transitorio, la de roedores agrestes peridomésticos, especialmente caviás, cricetidos y lagomorfos. El paso de la infección doméstica al campo, se hace por diversos animales, especialmente mustélidos, monodelfis, didelfis y conejos, relativamente insusceptibles a la infección, pero que debido a sus hábitos de alternar en nidos de roedores domésticos y campestres, transportan pulgas infectadas de los primeros a los últimos. El clima a campo abierto, solo temporalmente favorable a la *X cheopis*, y la ausencia de vector propio en las especies peridomésticas, da carácter transitorio a la epizootia. De otra manera, como en Argentina, por ejemplo, la peste no tardaría en hacerse selvática.

### LA PESTE SELVÁTICA

Comienza su historia americana en los Estados Unidos, hacia 1903, en el Condado de Contra Costa, California. En 1947, abarca 14 Estados incluyendo Kansas y Texas, en el área situada al oeste de los Montes Rocallosos, entre el meridiano 102° O y el Pacífico y entre los paralelos 30° y 52° N, en Canadá. Reconocida primero en el *Ostellus beecheyi*, hoy compromete 18 subespecies de ardillas de tierra, ardillas rojas y voladoras, el *chipmunk* de Tahoe, marmota de 2 variedades,

*cheopis*, aunque con mayor período de incubación extrínseca de la infección.

Eskey, Meyer, Wayson y otros, resumen así los hechos más impor-

ro gran in-  
a un mismo  
roedor, preferencias por hospedero o madrigueras, variable según las especies

(2) Susceptibilidad a la peste grande y casi uniforme para los diversos roedores, resistencia en ardillas, especialmente hembras, aumentada después de las epizootias; menor susceptibilidad de adultos supe

(  
espe  
biológicas, distribución geográfica  
que las ardillas de tierra y los *Cynomys* son actores primarios e independientes de la infección.

## PROFILAXIS DE LA PESTE

Después de las demostraciones que hemos hecho para el control de la peste murina en las ciudades de Tumbes, Huacho, Haciendas el Carmen y Laredo y Trujillo, todas ellas auspiciadas por la Oficina Sanitaria Panamericana en conexión con el Gobierno del Perú, podemos declarar enfáticamente que la peste urbana y la rural campestre son de fácil dominio mediante el uso conjunto de insecticidas de acción residual, especialmente DDT, y de rodenticidas de la eficacia del fluoracetato de sodio o 1080. En cambio, la profilaxis de las pestes agreste, silvestre y selvática, es un problema por resolver.

## CLÍNICA Y TERAPÉUTICA

En América se han observado ciertas formas clínicas de peste humana que no se mencionan en otros continentes, a lo menos con la viruela pestosa, fiebre mulosa y la forma endémica de peste ambulatoria. El tratamiento de la peste con estreptomycin, ensayado con esplendidos resultados en el brote de Buenos Aires, como el uso de las drogas sulfamidadas, especialmente sulfatiazol y sulfamerazina, han entrado en la rutina del tratamiento antipestoso.

Tanto la clínica, como la terapéutica de la enfermedad humana, carecen integralmente de significación epidemiológica en cuanto la peste del hombre es un mero accidente de la peste de los roedores. El dominio de la enfermedad humana, no debe hacer olvidar el verdadero camino de la profilaxis antipestosa.

TABLA 1 — Peste en América

País		Casos	País		Casos
A					505
P					1936
P					2
C					878
C					87
C					22,852
Ecuador					61
Costa		8,830	Trinidad		157
Sierra		1,472	Uruguay		143
Zona austral		1,614	Venezuela		

<sup>1</sup> Aproximados

## ROEDORES COMPROMETIDOS EN LA PESTE SELVÁTICA AMERICANA

- 1 Estados Unidos — *Sciuridae* *Citellus armatus*, *C. beecheyi beecheyi*, *C. columbianus*, *C. richardsoni*, *C. taylori*, *C. townsendi*, *C. variegatus grammurus*, *U. v. utah*, *C. washingtoni*, *C. w. washingtoni*, *C. w. loringi*, *Ictidomys tridecemlineatus*, *Tamias*

<sup>1</sup> Solo pulgas

chado las mesetas y laderas de los cerros para el sembrío de maíz, la peste ha repercutido en pseudoepidemias, porque en efecto se trata de casos que han recibido la infección sólo en los campos en que concurre epizootia de cricétidos y en los cuales, después de la muerte de los roedores se encuentra abundancia de vectores libres. El único vector reconocido hasta la fecha es la *Polygenus litargus*, a su vez espléndido reservorio de la infección por su gran resistencia a las condiciones climáticas desfavorables. Su potencial vector es a lo menos igual al de la *I. cheopis*, totalmente ausente en esta zona, al igual que las ratas domésticas. El índice pulido por ardillas y cricétidos, es elevado en la época lluviosa, casi nulo en la estación seca, pero durante esta las pulgas se mantienen en las madrigueras en proporciones enormes. Las ardillas no hibernan, las epizootias destruyen los ejemplares juvenes y dejan en los adultos una infección residual, o latente difícilmente reconocible, pero recuperable en cobayos. Es posible que los casos humanos observados en la zona en 1934-1939, 1942-43, estén en relación con un ciclo de la peste selvática de esta zona. De paso hacemos notar que el género *Polygenus*, está representado en Sudamérica por una veintena de especies púldas, la mayoría de roedores silvestres y que su importancia sería enorme si la peste selvática continua su extensión en el continente, ya que su distribución geográfica abarca de Venezuela a Argentina y de uno a otro océano.

El foco de peste selvática descubierto recientemente en Huanca

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ratas, ni *I. cheopis*. Tampoco hay ardillas. Los reservorios primitivos son exclusivamente cricétidos, especialmente *Akodon mollis orophilus* y *Oligoryzomys longicaudatus stolzmanni*. A título secundario se infectan *Sylvilagus audenbergi* (f) y *Cavia porcellus* o *aperea*. El principal transmisor es la *Trichopsylla (Pleochaetis)* sp. n. La *Polygenus litargus* y posiblemente otra *Polygenus*, juegan rol secundario. Las destructivas epizootias entre cricétidos hacen pensar que el reservorio pulido sea más importante. Igualmente, el *Sylvilagus*, entre los que se encuentra alto porcentaje de infección latente. El rol de las *Leptopsylla*, *Tiamastus*, *Gracopsylla*, *Hoplopsyllus*, *Odonopsyllus*, *Cediopsylla*, *Ctenidiosomus* *Neotiphloceras* que se encuentran en los roedores de la zona, no ha sido aun determinado.

La peste comienza con carácter rural, de arrollándose la epizootia en los trigales.

la peste humana

la invasión de l

humana adquiere caracteres hiperepidémicos, desaparecen las familias completas y provocando a veces el pánico segundo del éxodo de los habitantes de extensas zonas, con abandono de deudos y de enfermos, en escenas que reviven la Edad Media.



*Liamastus calicicola*

*Rhopalopsyllus* y *Polygenus* más importantes de Sud América encontradas en animales silvestres, especialmente roedores *R. australis* aus-

*samuelis*, *P. occidentalis*, *P. peronis*, *P. roberti*, *P. steganius*, *P. atopus*, *P. adelus*, *P. versuta*, *P. rimatus*, *P. tripus*, *P. litus*, *P. litargus*, *P. dendrobis*, *P. agilis*, *P. plaumanni*, *P. truncatus*, *P. pygaerus*, *P. pradori*, *P. platensis cisadinus*

(Fuentes, Costa Lima, Anduce, Eskey, Wayson, Jordan, Guimaraes Del Ponte y Riesel, investigaciones inéditas del autor)

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<sup>2</sup> En las referencias 10 14 y 18 se enciende abundante bibliografía sobre peste en las Américas. En los archivos de la Oficina Sanitaria Panamericana de Washington hay 23 Informes inéditos del autor sobre Epidemiología de la Peste en el Perú

*asciurus douglasii albelimbatus*, *Glaucomys sabrinus lasiurus*, *Futamias quadrivittatus frater*, *Cynomys gunnisoni zunienensis*, *O. leucurus*, *O. parvidens*, *Marmota flaviventris engelhardti*, *M f nosophora*  
*Cricetidae Microtus*, *Onychomys*, *Reithrodontomys*; *Sigmodon*;  
*Neotoma cinerea occidentalis*, *N fuscipes mohavensis*, *N lepida lepida*,  
*N l intermedia*, *Peromyscus truei truei*, *P t gilberti*  
*Heteromyidae Dipodomys ordii ordii*

*tralis*, *M. a. joannae*; *Graomys griseoflavus griseoflavus*, *G g centralis*,  
*Hesperomys murillus cordobensis*, *Lepus europaeus Lagostomus*  
*maximus* Infección experimental *Cavia* (3 especies); *Akodon areni*  
*cola hunteri Tympanoctomys barrerae*, *Ctenomys mendocinus*, *Rei*  
*thron don auritus auritus*, *Hesperomys sp*, *Oryzomys flavescens*, *Lagi*  
*dium viatorum*

(Fuentes De la Barrera, Savino, Alvarado, Uriarte )

3 Brasil—Infección temporal en roedores silvestres, no verdadera  
pesta selvática *Cavia aperca*, *Galea spixi*, *Kerodon rupestris* *Ory*  
*zomys intermedius*, *Cercomys cunicularius*, *Sylvilagus brasiliensis*  
(También el monodelphis *Peromys domesticus* )

4 Perú—*Cavia aperca*, *Sciurus stramineus nehouzi*, *Oryzomys*  
*xanthaeolus xanthaeolus*, *Oligoryzomys longicaudatus stolzmanni*,  
*Oryzomys ntidus*,<sup>1</sup> *Akodon mollis orophyllus*, *Rhipidomys equatoris*,  
*Sylvilagus andensis* (1)<sup>2</sup>

### PULGAS ENCONTRADAS EN ROEDORES SELVATICOS AMERICANOS

1 Estados Unidos.—Vectores espontaneos o experimentales de

<sup>1</sup> Solo pulgas.

<sup>2</sup> Clasificación dudosa.

## ECOLOGICAL STUDIES OF RODENTS IN RELATION TO PLAGUE CONTROL

D H S DAVIS, M A, *Plague Research Laboratory,<sup>1</sup> Department of  
Health, Union of South Africa*

### ENZOOTIC AREA

In South Africa, plague rolling plains of the inland. The escarpment 150 miles in the form of a loop, within which lie the semiarid expanses of the karroo, high veld, and Kalahari. These areas now form the enzootic plague region of southern Africa, some half million square miles in extent. Major sylvatic foci in the high veld (northwestern Orange Free State) and the karroo (Cape Midlands) were established in the early years of the century as a result of the carrying of plague inland from the ports infected during the last pandemic. The expansion of the primary foci has proceeded steadily, and it now appears that at least within the Union and the territories immediately adjoining (Basutoland, South West Africa, and the Bechuanaland Protectorate) further expansion is unlikely to take place (Davis, 1948a)

### ENZOOTIC FACTORS

The factors that permit the continued existence of enzootic plague in certain areas are beginning to emerge with greater clarity now that the boundary between the plague infected and plague free areas has become more or less apparent.

*Rainfall*—Enzootic plague is confined to the low rainfall region of the summer rainfall area. It is absent in the winter rainfall area of the southwest Cape. The 25 inch isohyet (mean annual precipitation) coincides closely with the eastern limit of enzootic plague in the southern Transvaal, Orange Free State, and eastern Cape Province, and with its southern and western limits in the karroo. The topographical factor, the rain catching ranges of mountains of the escarpment of the east, south, and southwest, determines the distribution of rainfall and may itself prove to be important as a faunal barrier. There are, however, grounds for believing that mean annual precipitation is more intimately related to the limits of the enzootic area. For example, in the latitude of Johannesburg (latitude 26° S) the 25 inch isohyet is a hundred miles west of the escarpment, and the intervening territory is plague free.

*Rodent species*—A study of the geographical distribution of burrowing rodents forming the primary reservoir of sylvatic plague gerbils and ground squirrels, in relation to the enzootic plague area provides further clues. The ground squirrel (*Geosciurus inauris*) is

<sup>1</sup> At Johannesburg

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**Enzootic factors and control**—The geographical distribution of *X. eridos* and *X. piri* is a useful biological indicator of the conditions in which *P. pestis* can be perpetuated, but it does not explain them. This is a matter for future experiment. This and the other factors appear to be broadly correlated with the enzootic area as reflected

### ECOLOGICAL STUDIES OF THE GERBIL (*Tatera brantsii*) RESERVOIR

**Hyperenzootic areas**—Within the sylvatic plague region there are certain areas in which transmission to man follows in the wake of an epizootic with greater frequency (Davis 1948a). The most important of these is in the maize growing districts of the northern Orange Free State. Much of our knowledge of the ecology of plague has come from intensive studies in this area (Pirie, 1927; Ingram 1927; Davis, 1939). I shall review briefly the results of various lines of investigation into the population dynamics of gerbil to bring out the essential features of the epizootic cycle as a basis for a consideration of the most appropriate control measures.

**Breeding cycle**—Study of the breeding cycle over one season showed that although pregnant animals may be found at all times of the

Burrow temperature varies from about 21° C (70° F) in summer to about 10° C (50° F) in winter and shows no diurnal fluctuation a few

three gerbil fleas are common to other small rodents of the open veld but are rarely encountered on domestic rodents. The domestic rat (*R. rattus*) rat fleas gerbils

distributed entirely within but not quite throughout the known plague area. The namaqua gerbil (*Desmodillus auricularis*) is distributed almost entirely within the enzootic area but is confined to the karroo and parts of the Kalahari. In the karroo, it replaces the common gerbil (*Tatera*) but overlaps it in the Kalahari. The common gerbils

the southwestern Cape, *T. brantsii* from the eastern karroo, the high veld, and Kalahari, and parts of Natal, and *T. schinzii* from the Kalahari and its borders, the bushveld of the northern Transvaal and from west to east across southern Africa from Angola to Nyasaland (Davis, 1948b). *T. afra* is not found in the enzootic area. *T. schinzii* has a wide distribution in southern Africa and is found in the Kalahari side by side with the third species *T. brantsii*. *T. brantsii*, however,

generally with the limits of the enzootic area, with two important

and no proof of rodent plague though it has been suspected from time to time. The cause of the "crash" in gerbil numbers has not been determined. There is a factor missing in this area, and that is the flea *Xenopsylla eridos*, whose chief host is *T. brantsii*, with which it is found throughout the enzootic plague region.

*Flea species*.—The flea fauna of *T. brantsii* in the hyperenzootic area of the northern Orange Free State consists of three species.

gerbils and  
the plague  
known enzo  
The namaqu  
*Xenopsylla*  
*X. eridos*

overlap, on the northern fringe of the karroo, where it gives way to the high veld grasslands.

borders of the Union. While the antiplague organization of the Department of Health covers the whole of the country, its main activities are concentrated in these hyperenzootic areas. The sphere of

It is simpler and more effective, though by no means easy, to eliminate the immediate rather than the ultimate source of infection to man. Sylvatic foci can be reduced or even wholly eliminated on a small scale, but the return from measures directed against domestic rodents and fleas, the immediate source of infection in the majority of

objective is the elimination of rats in farm buildings in the interim ad hoc

principles must be applied. Under South African conditions, in particular, simple cereal poison baits distributed in bait containers on the lines developed in Britain during the war have given better results and more effective and hence more lasting clearance. The domestic rat (*R. rattus*) is quick to utilize cover, and furthermore its normal food is often dry. The bait containers are used as regular refuges in a night or two, and soaked cereal baits are an added attraction.

The main principle of control is therefore to keep the sylvatic foci under surveillance and to eliminate them on a small scale where practicable, but to concentrate upon preventing infection from becoming established in close proximity to man.

#### ACKNOWLEDGMENTS

I have to thank Dr Botha De Meillon, of the South African Institute for Medical Research, for his valuable contributions to the flea survey of southern Africa. I am indebted to the Secretary for Health for permission to present this paper.

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houses or farm outbuildings from the bites of *X. brasiliensis* during or among domestic rodents.

at peak abundance (in houses) . . . . .  
discontinuously about a mile or two apart. The size of . . .  
varies from half a dozen to 50 or more individuals. Each colony in-  
habits a series of warrens. The breeding females remain closely at-  
tached to the warrens in which they brought forth their first litter;  
the parent warrens over . . . . .  
the parent warren and may dig themselves out or at a

distance. The movement of individuals brings each warren a con-  
stant movement during a night's activity. In consequence,  
the colony is not hun-  
gry, mainly by adult  
tervals, is sporadic  
conditions is pre-  
sented a few smouldering  
initial foci involves  
in various stages of  
plated colony to die  
is erratic, it may  
its course. Major  
Man is at risk of  
the major epizootic, which, in effect,

is . . . . .  
the period—The explanation given  
by which plague persists during the  
but warlike population holds  
infective in  
or in keeping  
gation of an

plague-infectious . . . . .  
goes through changes in virulence during the epizootic cycle.  
crucial. So far no solid evidence has come to light to suggest that  
strains of *P. pestis*, isolated from different sources and at different  
periods in the epizootic cycle, differ materially in virulence from one

aspects are . . . . .  
whole there . . . . .  
free State, in  
which plague is hyperendemic . . . . . occupy about  
one tenth of the 250,000 square miles of infected territory within the



Dr G GIRARD (France) Dr Macchiavello speaks of pastoral and sylvatic types of plague. This distinction may be important in South America, but in places like Madagascar, it would create some confusion. We have had a lot of plague and have been unable to distinguish between the two types. One might use

that the movements of rodents are different in cities than in rural areas. In rural areas, we have observed large migrations governed by the amount of food available. It is possible to follow the movements of rats by noting the spread of plague from one area to another. I find Dr Girard's observation regarding terminology extremely interesting. In South America we do not have jungle plague. I agree with Dr Girard, and should like to have a resolution placed before the Congress that a smaller commission be appointed to define the terms used to describe various type of plague.

Major General SOKHAY (India) We too have studied wild rodents as a source of plague. We have found them infected only at the periphery of a focus of infection. The channel is from domestic to wild rodents. Certainly in the Bombay area, plague is almost exclusively of domestic rodents.

## ABSTRACT OF DISCUSSION OF PAPERS BY MACCHIAVELLO AND DAVIS

Dr DAVID E. DAVIS (United States), commentator The papers concerning the ecology of plague have emphasized a number of problems related to the life history of the mammalian reservoirs. A knowledge of the seasons of the year during which reproduction oc-

the spread of plague Davis' paper mentioned the fact that the female gerbil remains within a limited area near the warren and that

home ranges of individual animals releasing the animal indicate the extent of movement. The extent of home range may also be studied by tracking rats in fresh snow and by feeding a dye which colors the feces. By these methods it was found that in Baltimore 90 percent of 119 recaptures of individual brown rats were within 60 feet of place of original capture. Similarly on a farm 69 percent of

also that the rate of movement of rats are limited to a

of the favorable environment forcing the mammal to search for a new home. If, for example, a shed is torn down, then the rats will have to find new shelter and may spread far and wide. A second cause of movement is pressure of population which forces surplus

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# LES VACCINS ANTIPESTEURS VIVANTS (VIRUS-VACCINS)

DR G GIRARD, *Chef du Service de la Peste à l'Institut Pasteur (Paris)*  
*Ancien Directeur de l'Institut Pasteur de Madagascar*

## PREAMBULE

Des vaccins vivants (virus vaccins au sens pastorien) ont remplacé depuis 1934 à Madagascar et à Java les vaccins tués dans l'immunisation de l'homme contre la peste, et la pratique s'en est étendue depuis 1940 à d'autres pays.

Cette évolution s'est produite voici 15 ans, sous la pression des circonstances épidémiques, elle s'est imposée devant cette constatation que la vaccination classique était à elle seule impuissante à réduire de manière sensible l'incidence de la morbidité pesteuse quand des facteurs locaux inhérents au mode de vie de populations encore trop peu évoluées rendaient à peu près inopérante toute entreprise de prophylaxie étiologique.

L'expérimentation avait enseigné des 1895 à A. Yersin, puis en 1903 à Kolle et Hetsch, à Kolle et Otto, en 1907 à Strong que certaines souches de peste dont le pouvoir pathogène était atténué, soit spontanément, soit à la suite d'artifices de culture, pouvaient être inoculées aux rats sans incident et leur conférer une immunité plus solide que celle engendrée par l'injection de vaccins tués. En 1908, Strong inocula ainsi 200 personnes à Manille avec la souche Maassen V sans réaction fâcheuse. Cet essai resta isolé. Il parut téméraire, les bases expérimentales en étaient fragiles, on pouvait se demander si un virus vaccin ainsi obtenu était définitivement fixe dans son comportement. La fabrication des vaccins tués qui, à ce point de vue, offrait toute sécurité, était au surplus d'exécution plus aisée. Yersin n'avait il

été fute qu'avec la plus grande prudence, en s'entourant de toutes les garanties possibles? Le précepte édicté par Yersin ne doit jamais être perdu de vue. Nous savons en effet que l'introduction sous la peau d'un bacille pesteux virulent est généralement suivie d'accidents graves qui reproduisent le type de la peste naturelle, comme l'attestent plusieurs accidents de laboratoire, sans remonter à 1824, époque où Ceruti ayant effectué en Egypte des inoculations de pus prélevé sur des pesteux, eut à déplorer 5 décès sur 6 personnes ainsi "immunisées".

Les virus vaccins E. V. Madagascar (Girard et Robic), et Tjwidej Java (Otten), grâce auxquels nous pouvons, avec un recul suffisant, porter un jugement objectif sur la nouvelle méthode d'immunisation, ont fait l'objet de publications auxquelles nous renvoyons le lecteur

(1) Nous n'en tirerons ici que l'essentiel indispensable à la facilité de notre exposé. La vaccination à Madagascar par le virus vaccin E V a été rapportée par nous même avec J Robic, avec des documents annexes des plus suggestifs, au 3eme Congrès de Médecine Tropicale d'Amsterdam en 1938 (2), puis dans une communication en 1942 qui tient compte d'une expérience plus étendue (3). Pour l'application du virus vaccin Tjirwidej à Java on se reportera aux publications de L Otten (4) et de W de Vogel (5).

Dans les limites dévolues à ce rapport, nous nous proposons

- (1°) De rappeler les bases expérimentales sur lesquelles nous sommes fondé à préconiser l'adoption des virus vaccins
- (2°) De définir les propriétés requises pour qu'un virus vaccin soit susceptible d'être inoculé à l'homme, quelle que soit la technique suivie pour son obtention : choix des souches, contrôle, conservation
- (3°) De préciser les indications de ce mode de vaccination ses avantages et ses inconvénients comparés à ceux des vaccins tués.
- (4°) De dresser un bilan statistique des vaccinations effectuées dans le monde au moyen d'un virus vaccin antipesteux
- (5°) De résumer à la lumière des travaux en cours les acquisitions nouvelles dont nous sommes redevables à l'étude des virus vaccins dans le domaine immunologique, ainsi que les perspectives d'avenir qu'ils nous offrent dans celui de la protection vaccinale contre la peste

### BASES EXPERIMENTALES

Si des vaccins tués manifestent une certaine efficacité dans la protection des souris et des rats contre l'infection pesteuse, ils sont totalement inactifs à préserver le cobaye. Par contre, une seule inoculation

également le cobaye est immunisé contre les piqûres de *X cheops* pestigènes. L'immunité est à la fois solide et durable, elle était encore évidente après une inoculation par

etude comparée des deux virus vaccins dont les souches avaient été

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Jav  
poi  
ont

comportement vis à vis des bactériophages spécifiques sont identiques.

b Les souches dites 'avirulentes' et vaccinales ne sont que des souches de virulence *affaiblie* (et non atténuée, ce qui signifierait qu'elles sont définitivement fixées dans cet état) La virulence de manda à être précisée suivant l'animal d'expérience, le mode d'inoculation, la dose, etc.

portées en d'un

dégradation pour des raisons qui sont encore mal connues. S'il s'agit de dissociation, nous ignorons son processus. Il semble toutefois que la multiplicité des repiquages à 37°, les variations de température auxquelles sont soumises ces souches en cours de transport ou pendant leur conservation interviennent pour une large part. Inversement il n'est pas impossible, par des artifices expérimentaux, de faire re-

ment pour les souches E V et Tjirwidej, lesquelles, expérimentées dans plusieurs laboratoires étrangers, se sont comportées différemment parfois, mais n'ont jamais nulle part manifesté un pouvoir pathogène supérieur à celui qu'elles avaient lorsqu'elles furent considérées comme avirulentes' (8).

c Une souche 'avirulente' peut être dépourvue de toute valeur antigène. Un tube entier inoculé sous la peau d'un cobaye ne lui confèrera pas d'immunité. Chaque souche a, sur ce point, son comportement propre. L'assertion de Strong n'a pas de portée générale.

d Il n'est pas de technique qui permette d'obtenir à coup sûr au départ d'une souche de peste virulente, un virus vaccin stable. Les cultures répétées à 37°, les cultures en milieux aérés préconisés par Devignat (9), les cultures en bouillon alcoolisé à doses progressives jusqu'à 5% (Hetsch) atténueront plus ou moins rapidement la virulence du bacille pesteux. Les repiquages mensuels à 20° sur gelose nutritive y parviennent plus lentement. Il nous fallut 5 ans de cette pratique pour notre virus vaccin E V. Cinq autres souches isolées de l'homme en même temps et repiquées dans les mêmes conditions parvinrent bien au stade de virus vaccin après des délais analogues mais se dégradèrent assez rapidement. La souche Tjirwidej de provenance murine, était devenue 'avirulente' après seulement 6 mois. Il semble démontré, par Otten, Jawetz et Meyer (10) que des colonies isolées d'une culture virulente ou avirulente ne présentent pas toutes des propriétés identiques et que cette méthode permettrait d'obtenir rapidement des souches de virus vaccins.

Nous avons estimé, d'après nos constatations expérimentales, qu'un

échangées entre Java et Madagascar révélait des différences intéressantes dans leur pouvoir immunisant. A dose égale, la souche E V protégeait le cobaye plus solidement que la souche Tjwidej, alors que c'était l'inverse pour le rat. Cette donnée amenait des 1937 Otten à formuler l'hypothèse qu'il y avait au moins deux 'antigènes' dans ces virus vaccins, antigènes inégalement repartis. L'un surtout actif chez le cobaye, l'autre chez le rat. Pour nous, une sanction pratique s'en dégageait, dans l'ignorance du comportement de l'homme à l'égard de l'infection pesteuse quant à son mode de réaction du "type cobaye" ou du "type rat", un vaccin devrait être aussi actif pour l'un et l'autre de ces rongeurs et l'association des deux virus vaccins E V et Tjwidej correspondrait à cet idéal.

Les récents travaux des auteurs américains dont il sera fait état plus loin semblent bien confirmer l'hypothèse avancée par Otten.

#### PROPRIÉTÉS DES VIRUS VACCINS CHOIX, CONTRÔLE, CONSERVATION DES SOUCHES, ETC

La première qualité à exiger d'un vaccin antipesteux vivant doit être son innocuité pour l'homme, et la démonstration n'en sera donnée que sur l'homme.

Sur ce point capital, nous pouvons dire après la vaste expérience dont nous bénéficions aujourd'hui et qui manquait à nos précurseurs que la prudence dont il ne faut jamais se départir ne doit pas se confondre avec la pusillanimité. Certaines objections, en apparence logiques, mais purement théoriques, ne tiennent pas devant la réalité des faits. Faut-il rappeler que Yersin dans l'essai qu'il tenta sur lui-même sans incident, se servit d'une souche de peste qui tuait encore 30% des rats? Le fait n'est pas pour nous surprendre. Mais laisser entendre qu'un virus vaccin antipesteux doit être dépourvu de toute virulence, ou plus largement, de tout pouvoir pathogène vis-à-vis des

de leur efficacité chez l'homme. R Strong estimait pour sa part qu'une souche de peste suffisamment atténuée pour ne plus tuer le cobaye à la

rapport

a Rien ne permet de distinguer, en dehors de l'inoculation à l'animal, une souche virulente de *Pasteurella pestis* d'une souche avirulente. Les caractères morphologiques, culturels, biochimiques, la



Il serait souhaitable de disposer de vaccin concentré et desséché, qu'il suffirait de diluer dans l'eau stérile au moment de l'emploi. Sous cet état, le virus vaccin devrait être moins sensible aux écarts de température que les suspensions salines. La technique reste à trouver. La dessiccation, congélation, sans addition de substances enrobantes, n'a pas entre nos mains réalisé cet objectif. Il ne s'agit pas de préserver la vitalité d'un nombre réduit de microorganismes, mais du *maximum* exigé par le principe même de cette vaccination.

Jamais un accident imputable aux virus vaccins n'a été rapporté avec E V et Tjiwidej, les réactions locales et générales sont légères et quand exceptionnellement elles provoquent un arrêt de travail, celui-ci ne dépasse pas 48h. Les tous jeunes enfants (1 à 2 ans) supportent parfaitement le vaccin E V.

#### DONNÉES STATISTIQUES

Près de 4 millions d'inoculations de vaccin E V à Madagascar de 1933 à 1947, 175 000 en Afrique du Nord et au Sénégal (1943-44), 2 millions avec le vaccin Tjiwidej en 1935 à Java. La pratique des virus vaccins a été adoptée par le Congo Belge (vaccin E V) l'Union

19) P

à Java

G W

Meyer) Il n'est pas venu à notre connaissance que celles qui ont manifesté le plus haut degré de protection chez les rongeurs aient été inoculées à l'homme sur une échelle assez large pour que l'on en tire un enseignement pratique.

#### DONNÉES IMMUNOLOGIQUES

Les facteurs de l'immunité dans l'infection pesteuse sont loin d'être élucidés (20). Mais les réactions provoquées chez les animaux comme le cobaye par les vaccins vivants, réactions inexistantes avec les vaccins tués, sont vraisemblablement à la base du processus qui semble être d'ordre cellulaire plus qu'humoral. On sait que les virus vaccins actifs tels E V, Tjiwidej, sont décelables pendant près de deux semaines dans certains organes comme le foie, la rate ou les ganglions après une injection sous-cutanée d'1 milliard de germes environ, et entraînent une prolifération lymphoïde (11, 12). Jawetz et Meyer font des constatations du même ordre avec leurs souches A 1122 et 14 (19).

4 ans ou 10 ans,  
de  
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virus vaccin pour être efficace chez l'homme devait posséder quelques caractères fondamentaux : maintien d'un certain degré de virulence attesté par son comportement chez le cobaye (8 11 12) persistance de toxicité des corps microbiens Jawetz et Meyer réservent sur ce point leur opinion (13)

En vérité chaque souche avirulente possède son individualité. Mais l'accord doit être unanime sur l'impérieuse nécessité de soumettre à un contrôle régulier toute souche de peste destinée à être utilisée comme virus vaccin dans l'immunisation humaine. La conservation sur gelose nutritive, à la glacière ( $+2$  à  $4^{\circ}$ ) les repiquages espacés (une fois par an) nous ont réussi pour la souche E V dont les caractères se sont maintenus intacts depuis 14 ans à Madagascar comme à Paris. Le passage par le cobaye avec récupération des microorganismes par l'ensemencement des tissus où ils sont décelables pendant plusieurs jours après l'inoculation de doses élevées est à retenir aux fins d'une régénération ou d'un renforcement éventuels des propriétés antigènes.

#### CONDITIONS D'APPLICATION

Un vaccin vivant doit être préparé suivant les besoins et ne peut pas être stocké. Nous avons estimé sa validité à 1 mois au plus, et à la condition que les ampoules soient conservées à la glacière. La teneur en éléments microbiens est susceptible de varier dans les plus

cer jusqu'à 3 milliards le vaccin E V sans réaction excessive. En eau salée physiologique (8 p 1000), la lyse du bacille pesteux est très lente : contrairement à ce qui se passe pour la plupart des microorganismes pathogènes et après 2 ans on peut encore trouver des germes repiquables dans des suspensions contenant 1 milliard au départ. Mais il importe d'inoculer le maximum de germes viables : aussi le délai de 1 mois a-t-il été fixé arbitrairement. Cet inconvénient majeur n'existe pas avec les vaccins tués dont des réserves peuvent être constituées. Le contrôle de ces vaccins est au surplus très rapide.

Nous pensons en conséquence, devoir réserver l'usage des virus

être fabriqué sur place, dans un laboratoire approprié et chaque lot contrôlé dans sa pureté, son innocuité et son pouvoir préventif. Ainsi comprise la vaccination massive pratiquée en milieu épidémique réduit de 80% au moins le taux de la morbidité pesteuse (Girard et Robic, Otten).

Une inoculation de rappel est conseillée avant ou pendant la recrudescence épidémique annuelle : la protection est acquise dès le 5ème jour et n'est pas précédée de phase négative (Grasset) (14).

# EXPERIMENTAL APPRAISAL OF ANTIPLAGUE VACCINATION WITH DEAD VIRULENT AND LIVING AVIRULENT PLAGUE BACILLI

K. F. MEYER, M. D., *The George Williams Hooper Foundation, University of California, San Francisco.* In cooperation with L. E. FOSTER, L. E. BAKER, H. SOMMER, and A. LARSON

could be secured to further the use of antiplague prophylactics

*Antigenic structure of Pasteurella pestis and host specificity of the antigenic fractions*—According to Schütze (1932), Gorokhov (1940), and Bhatnagar (1940), virulent and avirulent plague bacilli possess two antigens, one corresponding to the "envelope" and the other to the somatic substance. The heat-labile envelope antigen is developed best at 37° C, while the heat stable somatic antigen forms as well at 20° as at 37° C. Since the immunogenic activity of a number of cultures of avirulent plague bacilli in the guinea pig was found to differ significantly from that in the mouse, Otten (1936, 1941) and Jawetz and Meyer (1944) determining factor in the strains. With the isolation present in *P. pestis* by Baker et al (1947), an important aspect of the clarified. By extracting -70° C with neutral salt solution, water soluble and water insoluble antigenic components were obtained. The water insoluble fraction is toxic for mice and rats (L. D. 50.8 to 15 micrograms) but is nevertheless highly immunogenic for these animals. It contains



The protective efficiency is raised to the extent that the survival rate of mice and guinea pigs injected with the precipitated or oil water emulsion is at least double over the survival rate of those animals treated with the essentially soluble antigen. Noteworthy is the fact that in some experiments an oil water suspension of killed detoxified (with alcohol or formaldehyde), plague organisms conferred as high

1 infec  
These  
virulent

plague bacilli are capable of conferring protection on guinea pigs. Alum and oils likewise enhance the immunogenicity of plague antigens for cotton rats (table 1). Superior formation of protective antibodies and agglutinins in monkeys follows the inoculation with formalin killed plague bacilli precipitated with alum or suspended in oil water emulsions.

*Influence of temperature of incubation and method of killing of plague bacilli on immunogenic efficacy*—The antigen of prime importance in the protection of the Muridae, and in all probability of man, develops in cultures at 37° to 40° C. Until more convincing evidence has been obtained that a more potent antigen is formed

genic potency tests on mice have continuously demonstrated that a diversity of preparations of bacilli killed by various methods are highly protective, provided the fraction IB is present in adequate amounts. Antigenic activity is maintained in killed plague bacilli by

alcohol, ethyl  
irradiation  
Between 10

and 50 percent of the guinea pigs are also protected by these prophylactics. The survival rate of the experimental animals depends on the challenging infection, the mode of administration, and the number and virulence of the organisms introduced. As might be expected, exposure to plague infected fleas or intranasal instillation readily overwhelms the inadequate immunity generated in guinea pigs by prophylactics quantitatively low in residue antigens. The records in table 2, selected from a large series, amply demonstrate that poor antigens may be readily converted into powerful immunogenic preparations when incorporated into oil emulsions according to the method of Freund and Bonanto (1942, 1944) or precipitated with alum. On a comparative basis, suspensions of bacilli killed and detoxified with formalin or alcohol are slightly superior to those of bacilli killed with phenol or heat. If the physical or chemical treatment is not so severe as to denature the fraction IB and the residue antigen, the resulting prophylactics are potentially active. Contrary to the opinion of Otten (1936) and Sokhey (1949), solution of the problem of plague prophylactics does not require "a better way of killing cultures" to

insoluble fractions which protect guinea pigs but not mice and against severe infections

TABLE 1—*Immunogenic specificity of soluble fraction I and insoluble residue of different animal species*

Antigen	Species of animal and dosage				
	Guinea pigs		White rats		Cotton rats
	Mfg		Mfg		
Fraction 1A	1.5	* 0/20			
Fraction 1A+alum	1.5	0/20			
Fraction 1B	1.5	0/19	6/67	14/19	1/9
Fraction 1B+alum	1.5	1/20	67	16/18	6/10
Insoluble residue	2.5	10/20	85	7/20	0/10
Insoluble residue+alum	2.5	16/20	35	8/19	
Insoluble residue	12.5	13/20			
Insoluble residue+alum	12.5	19/20			
Formalin killed bacillary suspension	1.5	2/10	25	17/19	5/10
Formalin killed bacillary suspension+alum	1.5	8/10	35	16/18	9/10

\* Survivors: total number following subcutaneous challenge with  $>10^7$  M. L. D. of virulent *P. p.*

TABLE 1—  
12.  
15.

ever, with the aid of a special mouse protection test (Meyer and I 1918), an increase in the concentration of specific protective antibody has been demonstrated in monkeys after the injection of fraction but not after the administration of residue antigen. Similarly significant development of protective serum antibodies has been noted in the serum of volunteers inoculated in three doses with 2.5 mg. of

is equally indispensable in the protection of mice against plague is deemed advisable to measure the immunogenic potency of a plague vaccine with mice rather than with guinea pigs.

that alum (potassium aluminum sulfate) and oils from petroleum lanolinlike substances enhance the immunogenicity of an anti-

guinea pig, the animal uniquely susceptible to plague. He seemed to be very doubtful that favorable results could be obtained in man, because relatively much smaller doses are injected. Since the plague immunity mechanism of man has been found to be similar to that of the mouse, the doses of antigen required to protect mice are used for a simple calculation. To immunize a mouse weighing 20 grams, 20 000, 000 dead plague bacilli with an adequate amount of fraction IB are required. Sokhey (1913, p. 39) estimated the dose at 6 000 000 to 7,000,000 organisms. To immunize a man weighing 60 kilograms at least 36,000,000,000 to 60 000,000,000 dead plague bacilli would be required. The maximal dose employed in antiplague immunization is 15,000,000,000 bacilli, divided into 2 or 3 doses. The local and general reactions have been

teers injected with

definite in 5 of 10

volunteers injected with 3,000,000,000 organisms in 2 doses, only 1 formed serum antibodies. It is obviously impossible to correlate through intentional infections the presence of protective antibodies in the sera of inoculated persons with the degree of immunity to plague. However, experiments on animals seem to indicate that the protective index should be one half of the normal index in both mice and guinea pigs in order to obtain a high rate of survival from massive infections which are invariably fatal to controls. Moreover, the concentration of protective antibodies in the blood decreases within 2 to 3 months and the immunity to a fatal infection correspondingly declines. An

TABLE 4—Protective antibodies in human sera following booster doses of plague vaccine

Volunteer	Prophylactic and date of inoculation	Mouse protection index	Mouse protection index after inoculation			
			7th day	14th day	21st day	28th day
		24.2				15.1
		14.8	15.4	12.4		8.2
		16.3			10.0	15.0
		14.0	8.6	1.6		8.3
						10.3
		14.8	8.2	8.2		8.9
		15.8	12.1			14.7
		10.4	13.3	13.9		12.3
						7.6
		12.1	7.7			8.1
		20.8				10.1
		16.8	6.9	2.0		8.5
		15.1			13.8	16.6
		17.0	15.4	10.9		15.0
		12.3			13.8	11.4
		18.1	14.0	10.3		10.8
		20.8	14.7			21.2
		17.7	13.2	13.6		15.7
		20.8				16.3
		14.5	13.4	10.6		9.8
		16.0				19.6
		14.0	2.0			2.1
		8.9	2.7	1.8		3.6
		14.2				14.7
		14.4	2.8			0.0
		9.2	4.7			1.7

hold the antigens intact but rather requires purification, detoxification and concentration of the effective immunogenic antigen. The mouse protective dose of soluble fraction 1B, detoxified with dilute acid or

antigen retained at the site of injection is slowly released and the immunisatoric stimulus is therefore prolonged

TABLE 2—Percentage of guinea pigs immunized with killed *P. pestis* antigen with and without adjuvants (15 mg in 2 doses) surviving subcutaneous infection with 600,000 *P. pestis* (Shasta) or exposed to infected fleas or to intranasal infection with 38,000 *P. pestis* organisms

Antigens	Subcutaneous infections	Exposed to infected fleas	Intranasal infection
Phenol killed	0/10 (0 days)	0/10 (0 days)	0/10 (0 days)
Formalin killed and alum	8/11-80 percent	9/19-47 percent	8/8-1 percent
Formalin killed and Falco	10/10-100 percent	14/15-93 percent	9/10-90 percent
Controls	0/10 (6-6 days)	0/20 (6-7 days)	0/10 (3-4 days)

\* Difference from 10 or 20 lost from anesthesia or nonplague death

TABLE 3—Relation of dosage to immunogenic effect in guinea pigs

Antigen	Dosage of antigen in 2 doses	Survival
Alcohol-acetone or formalin killed	12.5	96 to 100
Alcohol-acetone or formalin killed	2.5	70 to 80
Formalin killed	1.5	60
Heat-killed	1.5	61

*Dose of antigen and duration of immunity*—Use of plague prophylactics which contain between 10,000,000 and 20,000,000 killed bacilli,

least 25,000,000,000 dead plague bacilli precipitated with 12.5 milligrams of alum must be used in order to protect a guinea pig weighing 400 grams. Kolle, as early as 1903, emphasized the fact that very large doses of the plague organisms are required to confer immunity on the



TABLE 5—*Relationship between fraction IB and immunogenic power of avirulent strains*

Avirulent strains	Fraction IB in culture grown at 37° C Killed with cold acetone	Plague bacilli protecting 50 per cent of the mice against stand and infection	
		Total count	Viability count
No 1122	Percent 15-18	Number 300	Number 123
E V 76 (Girard)	5.0	850	170
Soemedang	1.7	120	105
53H 1 (Sokhey)	3.1	500	250
Java	2.3	17 000	7 100
Bombay	8.8	50 400	19 000
Belgian Congo 343	3.1	20 000	9 900
Tj (wide)	2.0	58 000	15 400
(E V 76 old)	3.5	74 000	18 000
K120 South Africa	1.9	109 000	21 000
No 14	0.5	90 000	30 000
TRU	0.2	490 000	171 500
	0.2	1 400 000	420 000
	0.2	8 000 000	3 690 000
	0.2	10 000 000	5 100 000
	0.2	20 000 000	15 900 000

† Intraperitoneal

\* Subcutaneous

TABLE 6—*Protection conferred to guinea pigs through avirulent plague vaccine in dosage of 3 billion*

Avirulent strains	Vaccine dosage	Survival after challenge		Persistence of viable or germinal on last day tested
		Total num ber of sur vivors	Percentage	
No 1122	300,000,000	18/20	90	21 days
	30,000,000	9/20	45	
	3,000,000	6/20	30	
	300,000	6/20	30	
	3,000,000,000	11/16	68	
E V 76 (Girard)	50,000	3/3	100	21 days
	500	3/4	75	
Soemedang	3,000,000,000	12/12	100	
53H 1 (Sokhey)	3,000,000,000	20/20	100	
Java	3,000,000,000	19/19	100	
Bombay	3,000,000,000	18/20	90	13 days
Tj (wide)	3,000,000,000	18/19	95	
(E V 76 old)	3,000,000,000	16/20	80	
K120	3,000,000,000	2/20	10	
No 14	"	20/20	100	9 days (none 14 days)
	"	17/19	90	
	"	14/20	70	
	"	15/20	75	
	"	14/19	74	
	"	1/20	5	9 days (none 14 days)
	"	1/20	5	

— Effect of the immunogenic action on mice and  
(table 5) disclosed  
the different aviru

lent strains grown at 37° C and killed with cold acetone. In com  
parative protection tests on mice and guinea pigs the immunogenic  
power of the different strains was evaluated. Dilutions of agar cul

experiment on mice exposed to infected fleas 1 to 4 weeks after the last inoculation of antigens demonstrated that the duration of the

(7 000 000 000 to 60 000 000 000 bacilli), a series of primary inoculations and frequent reimmunizations is absolutely essential

*Revaccination and individual response to plague vaccination.*—The duration of immunity and the effect of booster doses have been tested by measuring the specific humoral antibodies found in the blood of volunteers before and after antigen inoculations. The data in table 4 show that the serum protection index of the majority of individuals previously inoculated with antigens or avirulent plague bacilli and then reinoculated rose to a normal level. Without exception the sera

was prompt, within 7 to 14 days. In fact in some instances although protective antibodies had not been produced after the first injection they were produced after the second. In some instances (4 of the 10) despite repeated stimulation protective antibodies were not formed

volunteer had such a concentration of antibodies in his serum that 0.5 milliliters protected each of 10 mice against 100 M. L. D. of virulent plague bacilli. On the basis of the results of the serum protection tests and on the assumption that these tests yield a reliable index to an individual's quality and degree of immunity it may be concluded that (1) killed suspensions of plague bacilli are immunogenic for man, (2) the customary antigenic doses of prophylactics without synergists administered in the past are inadequate but the deficiencies may in part be overcome by repeated inoculations, (3) revaccination constitutes a reliable method of establishing and of renewing immunity, and (4) in some cases reinoculation fails to enhance the degree of immunity.

*The immunogenic activity of living avirulent plague strains.*—The impressive results of large scale antiplague vaccinations (Otten 1941, Girard and Robic 1936 and 1942, Grasset 1946) were obtained with different strains of *P. pestis* made avirulent through a variety

of the mice, 100,000,000 -- -- -- 1 cc. 100 percent of the guinea pigs 1 cc. 100 percent required to accomplish a dose of 50,000,000 organisms of the strain Soemedang immunized only 55 percent of the house rats (*R. diardii*), in its present state, although low

in its wanderings through different laboratories has lost its main characteristic, the ability to produce necroses in the spleen and liver of guinea pigs and mice. Whether this deterioration is in some way connected with the partial loss of the toxin and fraction IB has not as yet been determined. A transplant of the strain obtained through the courtesy of Dr. G. Girard directly from the Pasteur Institute behaves

it is rich in fraction IB, and of low toxicity, and relatively few bacilli immunize mice. Provided the findings with dead and living plague bacilli obtained from experiments on animals reflect the mechanism of plague immunity in man, and provided the results of large scale vaccinations both in Java and in Madagascar are acknowledged as valid, it may be concluded that avirulent strains possessing the two major antigens in proportions equal to that of virulent plague strains will serve as effective prophylactics. The isolation, evaluation, preservation, and distribution of such avirulent strains is one of the most urgent and important problems in the prevention of plague. Author

shadows the recognized fact that the organisms invade the tissues in the tissues. It is recalled that Otten (1936) found the Tjirwidj strain in the spleen for 7 days, and the Java strain for 11 days after subcutaneous injection. Jawetz and Meyer (1944) made similar observations with strains 1122 and 14, and Girard and Radoudy Ralatsy (1940) found the E. V. strain for 11 to 13 days in the spleen and

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tures examined for total bacterial count and plated on blood plates for viability were tested on a series of mice. The number of dead

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injected subcutaneously. A dose of 1,000,000,000 was chosen, since Otten (1941) had demonstrated that a vaccinating dose of  $\frac{1}{8}$  agar slant (2,500,000,000 organisms) of his strain Tjirwidej gave a survival rate of 82.8 percent in mice and 95 percent in guinea pigs. Quantitative evaluation of the smallest number of avirulent plague bacilli capable of protecting guinea pigs, as already presented in the papers by Otten, was not deemed necessary. The strain 1122 (3,000,000,000 organisms, 1,800,000,000 living) used in a vaccine not only stimulated the appearance of serum agglutinins (1:32 + + + +) and significant concentrations of mouse protective antibodies (27 to 87 protection index) in 3 of 4 monkeys but also the animals resisted a challenge of 100,000,000,000 organisms of the same strain 1122 on the eighth day after injection.

On the other hand, in 10 volunteers injected with the Tjirwidej strain and strain 14, at the same time in the same dose, protective serum antibodies were not found, although these

antibodies were not found in the serum of the volunteers.

and most of the volunteers were not protected.

Furthermore, one must share with Otten and Girard the deep concern that dissociation of highly immunogenic avirulent strains is one of the disadvantages which constantly threatens their use as plague prophylactics. For example, strain Tjirwidej, now available, apparently underwent deteriorating changes. According to Otten (1941, p. 80), 0.000001 milliliter of broth culture protected 60 percent

Chemically killed detoxified (with formaldehyde or alcohol) virulent or avirulent plague bacilli grown on agar at 37° C precipitated with alum may replace living plague bacilli in vaccines. Ease of preparation and distribution, as well as safety of administration has recommended vaccines made with the former to the Army and Navy of the United States and the public health workers in India. Purified atoxic fraction IB antigen in a particulate form stimulates excellent antibody production in man. The preparation of this antigen is difficult and expensive. Prophylactics made from dead bacilli have a limited range of practical application without the administration of large doses and frequent booster doses (at least every 3 to 6 months). A dependable degree of protection can only be maintained with killed plague organisms provided large doses, preferably precipitated with synergists, are injected at frequent intervals.

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## ABSTRACT OF DISCUSSION OF PAPERS BY GIRARD AND MEYER

Dr A. FELIX (United Kingdom) I should like to ask Dr Girard whether he has noted a decrease in the case fatality rate corresponding

Dr Felix inquired whether the vaccines had been examined also in passive protection tests. Such tests he thought might perhaps disclose a harmful effect of one or the other of the chemical agents

smears prepared from the liver. Wherever the E V 76 avirulent *P. pestis* multiplies it produces focal necrosis and the site of this necrosis

vaccines involves some risk. However it is generally recognized that invasiveness, multiplication and persistence of attenuated or modified viable bacteria and viruses (used in anti-tuberculosis, anti-Bang vaccination, anti-smallpox and yellow fever vaccines) are collectively responsible for the high antigenic power they exert in contrast with the treated infective agents. There is every reason to believe that the high degree of immunity is dependent upon persistence of the

that the treatment required to produce killed organism prophylactics altered the antigens. The observations previously detailed clearly show that the crystalline fraction IB and residue in adequate doses or

20 gram mouse. Since approximately 1 000 bacilli with 200 viable elements of strain 1129 protect 50 percent of the mice it is obviously essential that a ten thousandfold multiplication in probably over 100 or more generations produces the required amount of antigen. Finally it must not be overlooked that plague bacilli are principally phagocytized by the reticuloendothelial cells. In the living state they are brought into more intimate contact with these cells and leave just as in tuberculosis and *Brucella* infections more permanent imprint on them and their descendants than do soluble nonparticulate antigens.

#### SUMMARY AND CONCLUSIONS

Experimental and theoretical considerations fully support the conclusions based on the excellent field experiences with single doses of vaccines composed of living avirulent plague bacilli. In endemic areas where native populations are heavily exposed a one dose plague prophylactic has administrative and economic advantages. The avirulent plague bacilli must possess the same antigenic make up as the virulent strains and must be of proved immunogenic power both for mice and for guinea pigs. Antigenic potency must likewise be evaluated on the basis of study of immunization of volunteers with

which is convenient for extended transportation without refrigeration and of safe administration must be developed.

## SULPHONAMIDES AND ANTIBIOTICS IN THE TREATMENT OF PLAGUE

S S SORNEY, and P M WAGLE, *Haffkine Institute, Bombay*

The evaluation of therapeutic agents in plague is a comparatively easy matter. As plague is essentially a disease of rodents and

experimental procedure in a previous paper (1) we used the Institute inbred white mouse weighing 21-30 grams as the test animal. This animal is highly susceptible to plague, subcutaneous

4 to 8 days

drying from

virulence undiminished almost indefinitely, we have now used the same dried culture for over 7 years.

With the availability of such a laboratory test, we have tested out the given therapeutic agent in experimental infection making clinical trials. In our studies extending over several years the results of the tests in experimental infection have always paralleled those of clinical trials.

In this paper we give the results of trial of sulphadiazine, streptomycin, and streptomycin in both experimental infection and human disease and also of tests of sulphamethazine and penicillin in experimental infection.

### TESTS IN EXPERIMENTAL INFECTION

In human bubonic plague, the most important factor which determines the issue is the development of septicaemia. In mice, under the experimental condition indicated above, septicaemia develops from 24 hours to 72 hours after the induction of infection by the subcutaneous route. By 48 hours about 50 percent and by 72 hours 100 percent of the mice develop septicaemia. In experimental infection, therefore, we start treatment with the therapeutic agent under trial in one of two batches 48 hours and in another batch 72 hours after the induction of infection. It may be added here for record that if the treatment is started at the

*similar to the alteration due to the formalin treatment of the typhoid bacillus which leads to the well established "functional deficiency" of the V<sub>1</sub> antibody (Felix, A, and Bhatnagar, S S, 1935, Brit J Exp Path, 16, 422)*

Major General SOKHEY (India) We in India have used vaccines made of killed organisms and on the whole the results have been good I believe we have reached the point where the relative merits of the two types of vaccines should be decided in the field

Dr MACCHIAVELLO (Peru) I agree with General Sokhey that a field test of vaccines is in order Dr Girard seems to imply that vaccination largely solves the problem of plague In the Americas, vaccination is not the solution, primarily because it necessitates a tremendous amount of work for relatively few cases

Dr GIRARD (France) In Madagascar it has been our experience that case mortality and case morbidity are essentially synonymous The disease is most often diagnosed after death

largely of protein, and there is very little carbohydrate We are certain that killed antigens are highly efficacious I don't think there is any great difference between heat killed and chemically killed organisms We have not yet performed a sufficient number of protection tests, but plan to carry out more.

I agree with Dr Macchiavello regarding the widespread use of vaccines in areas of low incidence of plague In California we have seen but one case since 1942 Certainly we are not justified in vaccinating the population under these circumstances



of the drugs in blood achieved with this dose will be described in another paper

TABLE 2—*Results of treatment of experimental plague in mice with penicillin and streptomycin*<sup>1</sup>

	Period elapsing between infection and drug administration	Number of organisms used in test infective dose	Mice used	Deaths in mice up to 31 days after infection	Controls	
					Mice used	Deaths
Penicillin	Hours 48	142	Number 10	Number 10	Number 10	Number 10
Streptomycin	48	142	10	0	10	7 5 3
Streptomycin	72	124	10	1	10	(3.5) 10 (5.0)

<sup>1</sup> Each drug was given by mouth in a dose of 5 milligrams 4 times a day for 5 days

<sup>2</sup> The figure in parentheses in the last column denotes the average duration of life of the controls

These experiments show that both streptomycin and sulphonamides have a very remarkable curative action in plague, but the action of streptomycin is definitely superior to those of sulphonamides

### CLINICAL TRIALS

About the middle of November 1947 an epidemic of plague broke out in a group of villages some 40 miles away from Poona. A temporary hospital with laboratory facilities was organized to test

journey on rough roads

plague. One case showed definite primary infection of the lung. The treatment was started with streptomycin and sulphadiazine. The two drugs were given in strict rotation. No selection of cases was made, but from the one hundred and thirteenth case we changed over from sulphadiazine to sulphamerazine.

*Plan of trial*—On admission every patient was examined for a bubo and other clinical signs of plague. Before any treatment was given, the bubo was punctured and the sucked up fluid in the syringe was plated on a blood agar slope. Also, 0.5 milliliter of blood was

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the mice were kept in batches of 5 in special cages designed for experimental work in tropical countries (2). The cages were housed in an air conditioned room with a temperature of 75° to 78° F.

The mice were observed for at least 31 days. They were examined once a day and deaths were noted. Those dying within this period were dissected and examined for evidence of plague. The surviving animals were similarly examined.

The sulphonamides under test, i.e., sulphadiazine, sulphamerazine, and sulphamethazine, were made into an emulsion with 10 percent solution of gum acacia so that 0.5 milliliter of the emulsion contained the selected dose. This quantity of the emulsion was introduced with a pipette into the stomach of the mouse. The drugs were administered

penicillin and streptomycin were administered subcutaneously, the selected dose being contained in 0.2 milliliter of the solution. Penicillin was administered in a dose of 1,000 units four times a day for times a day or the next 5 were helped by the work reported before (3-5)

and 10 milligrams four times a day for periods varying from 3 days to 10 days. The dose of 5 milligrams four times a day for 5 days appears to give the best results. Both sulphadiazine and sulphamerazine in the larger dose seem to be toxic to mice. The concentration

TABLE 1—Results of treatment in experimental plague in mice with sulpha drugs<sup>1</sup>

	Period elapsing between infection and drug administration	Number of organisms used in test infective dose	Mice used	Deaths in mice up to 31 days after infection	Controls	
					Mice used	Deaths
Sulphadiazine	Hours	144	Number 10	Number 6	Number 12	Number 12
Sulphamerazine	45	144	10	1	12	(5.7) 12
Sulphamethazine	45	144	10	3	12	(5.7) 12
Sulphadiazine	72	144	10	6	12	(5.7) 12
Sulphamerazine	72	144	10	3	12	(5.7) 12
Sulphamethazine	72	144	10	3	12	(5.7) 12

<sup>1</sup> Each drug was given by mouth in a dose of 5 milligrams 4 times a day for 5 days.

<sup>2</sup> The figure in parentheses in the last column denotes the average duration of life of the controls.

If to the results of the present clinical trial we add the results of some previous clinical trials (6) and (7), we get a more comprehensive picture. Results are given in table 4. In this table we also give the figures for controls from a previous trial.

Though the results obtained with the different treatments do not

respectively to reach normal temperature. In severe cases, with septicaemia at the time of the commencement of treatment, streptomycin brought down the temperature to normal on an average in 55 hours while sulphadiazine and sulphamerazine took 85 hours and 89 hours respectively to restore the temperature to normal.

TABLE 4—Results of all cases treated with or without septicaemia at the commencement of treatment

Cases	Treatment	Number of cases	Number of deaths	Case mortality
With or without septicaemia...	Streptomycin	124	8	Percent 6.5
	Sulphadiazine	168	16	9.5
	Sulphamerazine	149	9	6.0
	Controls	163	96	58.1
	Streptomycin	30	3	10.0
With plague septicaemia	Sulphadiazine	91	13	14.3
	Sulphamerazine	22	7	31.8
	Controls	91	84	92.3

No serious toxic symptoms were noticed with any of these drugs. In the case of streptomycin, two cases developed mild temporary psychosis and one case dermatitis which disappeared with the stoppage of the drug. In the case of sulphamerazine one case developed severe dermatitis which disappeared when the drug was stopped. No other symptoms were noticed.

One case of the present series had primary infection of the lung pneumonia, streptomycin was given. The case recovered. The drug was administered for 10 days.

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important factor which decided the issue in bubonic plague was the development of septicaemia. If the lymph gland prevented the spread of the infection to the blood stream and the infection remained localized, spontaneous recovery took place in a large percentage of cases, but if septicaemia developed, the infection proved fatal in over 90 per cent of the cases.

**Dosage Streptomycin**—In relatively mild cases, an initial dose of  $\frac{1}{2}$  gram followed by  $\frac{1}{2}$  gram every 4 hours was given until the temperature remained normal for 24 hours. In severe cases, 2 $\frac{1}{2}$  gram of the drug every 4 hours was given until the temperature remained normal for 2 to 3 days. Thus, in mild bubonic cases, without septicaemia, the total quantity of the drug used varied from 4 to 6 grams. In septicaemia cases, the total quantity of this drug used averaged 6 to 12 grams, but in five cases of exceptional severity as much as 25

rose to about 20 units and more per milliliter of serum. This estimation was made only in a small number of cases.

**Sulphadiazine**—An initial dose of 4 grams was followed by 2 grams 4 hours later. Thereafter, 1 gram was given every 4 hours until the patient's temperature remained normal for 2 days. This dose maintained a concentration of 10 to 15 milligrams per 100 milliliters of blood.

**Sulphamerazine**—An initial dose of 4 grams was followed by 1 gram 4 hours later. Then 1 gram was given every 8 hours till the temperature remained normal for 2 days. This dose maintained a concentration between 10 to 20 milligrams per 100 milliliters of blood.

**Results**—Of the 277 cases of plague, 18 cases were already on the

phamerazine. We exclude these 34 from our total of 277 cases of plague and give the result of the treatment of 243. We may add here that of the moribund cases, 6 were treated with streptomycin and 8 were treated with sulphamerazine but they all died within 15 hours. The results of treatment are given in table 3.

TABLE 3.—Results of all cases of plague treated with or without septicaemia at the commencement of treatment

Cases	Treatment	Number of cases	Number of deaths	Case mortality
With or without septicaemia.	Streptomycin	124	5	Percent 4.0
	Sulphadiazine	47	2	4.3
	Sulphamerazine	72	6	8.3
With septicaemia	Streptomycin	20	2	10.0
	Sulphadiazine	11	2	18.1
	Sulphamerazine	13	9	69.2

2° En cobayos que recibieron inoculación de pequeñas dosis de bacilo pestoso, se inició el tratamiento después de 24 horas con dosis de 5,000 unidades de estreptomycina cada tres horas con un total de 135,000 a 225,000 unidades. Los animales sobrevivieron desapareciendo los síntomas de infección que se iniciaban. Los controles murieron entre las 48 y 96 horas con septicemia pestosa.

3° Cobayos que, después de inoculación de gérmenes virulentos, recibieron solo una dosis de estreptomycina de 10,000 a 20,000 unidades, presentaron síntoma de infección, muriendo después de 7 y de 9 días los animales que quedaron con este solo tratamiento. Pero sobrevivieron los animales que después de tres días de la inoculación continuaron recibiendo tratamiento fraccionado, como refuerzo.

4° Contactos previos "in vitro" de suspensión de bacilos pestosos y de 20,000 unidades de estreptomycina por el tiempo de 10 a 35 minutos, fueron inoculados a grupos de cobayos. Todos quedaron protegidos y los controles murieron.

#### Conclusión

Como experimentos de laboratorio dieron resultados muy satisfactorios, que iniciarían el tratamiento eficaz de la peste mediante la aplicación metódica y oportuna de la estreptomycina. Que es lo que comunico en esta sesión.

Dr. MELFVEY (United States) I should like to ask Dr. Sokhey whether he has used streptomycin and sulfonamides in combination.

General SOKHEY (India) Our experience with serum has been entirely satisfactory. I agree with Dr. Girard that serum and sulfonamides together quickly bring the disease under control. But serum is expensive, and we try to do without it.

In our studies, we have not used a combination of sulfonamides and streptomycin. We have sought only to study the efficacy of the various drugs independently of one another.

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## ABSTRACT OF DISCUSSION

Dr GIRA " " " "  
 mycin for  
 does Gene  
 combine sulfonamides and serum?

Dr FELIX VEINTEMILLAS (Bolivia) Refiriendome a los estudios publicados en Julio de 1947 en la revista "Suplemento del Instituto" sobre esta materia, y que se hicieron a cargo del Dr. Jose ...  
 is allí  
 para  
 llo en

cultivos y como protector y curativo "in vivo" ante la inoculación en cobayos

## In vitro

1º Sobre placas de Petry con agar, agar sangre o suero se sembraron diferentes cepas virulentas de bacilo pestoso poniendo la superficie del medio en contacto con diluciones de estreptomycina desde 10,000 unidades a 1,250 unidades y sus respectivos controles. Mantenidos en la estufa a 37 grados centígrados, la observacion de los resultados fué de negativo desarrollo bacteriano para los contactos de bacilos y de estreptomycina y muy positivo para los controles

2º Tubos conteniendo 1 cc. de caldo sembrados de peste y puestos en contacto con diluciones de estreptomycina desde 5,000 unidades hasta 0,14 unidades dieron desarrollo bacteriano negativo ante las diluciones del antibiótico mayores a 20 unidades y positivo con las menores a esta dosis. En estos ultimos cultivos en que hubo desarrollo de los bacilos pestosos, estos presentaban formas de involución y de cambios morfológicos, ante el examen microscopico

## In vivo

Se dispuso de lotes de animales sensibles al bacilo de la peste para los experimentos que se mencionan.

1º Varios cobayos que, luego de ser inoculados con dosis mortales de bacilo pestoso, recibieron cada tres horas diurnas, en 3 días un total de 54,000 a 135,000 unidades de estreptomycina. Todos los animales sobrevivieron despues de una observacion de 30 días, muriendo los controles en 24 y 48 horas de septicemia pestosa

This was first shown by the early work of Hadley (1925-26), Burnet (1927, 1929) and Levine and Frisch (1934), who demonstrated the relationship between *Salmonella* bacteriophages and certain O antigenic components. As soon as the so-called V<sub>1</sub> antigen of the typhoid bacillus was discovered (Felix and Pitt, 1934) a number of investigators, working independently of one another in different countries

it is related, to

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*Salmonella*

phages because of the more limited distribution of the common O antigens in the various *Salmonella* species, but there is overlapping even with V<sub>1</sub> phages. However, the most important difference between the two is the peculiar adaptability of anti V<sub>1</sub> phages, first observed by Craigie and Yen.

There is an abridged version of the scheme now in use for the typing of typhoid bacilli. It shows only 15 different types but in reality the number of well defined types and subtypes is at present 24. The number will certainly increase very soon, when typing is adopted in other parts of the world.

All the type phages have been derived from one single strain of V<sub>1</sub> bacteriophage by a process of adaptation, and we must assume that the large V<sub>1</sub> antigen molecule of the typhoid bacillus contains at least 24 different determinant groups or bacteriophage receptors. These cannot be detected by any of the customary serological methods, only

the theoretical point of view, but there is no time to discuss it now. For practical purposes the important fact is that the V<sub>1</sub> phage type of a strain is a permanent character, and that typing of the typhoid

as those obtained in  
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in the former is

The typing scheme for paratyphoid B bacilli was developed in a similar manner. Six distinct types and subtypes of the paratyphoid B bacillus have been identified so far by means of adapted V<sub>1</sub> phage preparations. Again, the first place in the scheme has been assigned

## Session 4 ENTERIC DISEASES, CHOLERA, ELECTRON MICROSCOPY

*Friday, May 14—9 30 a m to 12 m*  
*Departmental Auditorium, Room B*

### MODERN LABORATORY METHODS IN THE CONTROL OF THE ENTERIC FEVERS

A FELIX, *Public Health Laboratory Service, Central Enteric  
Reference Laboratory, London*

Two recently introduced laboratory methods have proved to be of great service in the control of the typhoid and paratyphoid B bacilli by means of Vi agglutination tests as an aid in the detection of typhoid and paratyphoid B carriers.

These two laboratory techniques, along with the introduction of refined culture media, have provided the epidemiologist and public health administrator with new weapons to fight the chronic carrier. Since every chronic carrier represents a potential focus of the infection, it is obvious that the attainment of the ultimate goal, namely, the eradication of the enteric fevers, depends on the control of the chronic carrier.

#### TYPING OF TYPHOID AND PARATYPHOID B BACILLI BY THE VI BACTERIOPHAGE

Until 1938 the typhoid bacillus had been regarded as a single species that could not be further subdivided. In 1938 Craigie and Yen made the important discovery that the typhoid bacillus could be subdivided into two distinct types by the use of the Vi bacteriophage.

summarized herein

ter  
of



There was a slight increase in the proportion of untypable strains in Britain during the last 2 years. This is due to the return home of demobilized service personnel and to increased travel facilities since the end of the war, as a result of which hitherto unknown phage types

would appear that the necessity for using the typing method in every case. When a typhoid outbreak of considerable size occurs, it is usually not difficult to detect the car

In Britain the incidence of typhoid and paratyphoid fevers has been very low during the past few years.

In regard to the total notifications of typhoid and paratyphoid fevers in England and Wales, that is, in a population of about 43 million, there was a marked increase in the incidence of enteric fevers was due to numerous outbreaks which were of fairly large size. The incidence was very low indeed except for 1946, when our statistics were again spoiled through 2 outbreaks of paratyphoid B and 1 outbreak of typhoid fever, each comprising about 200 cases.

Because of the low incidence of the disease, it was considered opportune to organize a so called Central Enteric Reference Laboratory and Bureau to serve for the whole of Britain, i.e., for a population

several counties and many administrative districts. A number of epidemiological investigations conducted on a nation wide scale were only made possible owing to the fact that they were based on information accumulated in the records of the central laboratory.

Two spot maps were made of the County of Devon, a rural area in southwest England, to illustrate the great advances that has been made through the application of Vi phage typing (J. C. Cruickshank 1947). The County of Devon is one of the largest administrative areas in rural England, about 2,600 square miles with a population of half a million.

The second map showed that these cases and carriers are

to the type of bacillus that is lysed by all the typing phages; the remaining types are characterized by their particular sensitiveness to the homologous adapted type phage. On the other hand, anti O

typhoid A bacilli by a similar procedure. The preliminary results are very satisfactory, and a typing scheme is now being developed in collaboration with Professor R. G. Dhayagude and Dr. D. D. Banker of Bombay.

Russia in 1918, is a highly virulent  $V_1$  positive form employed as vaccine strain. Strain H901, isolated at the same time and place, is a  $V_1$ -negative form of low virulence. Strain O901, a permanent nonmotile

negative variant after an interval of 18 years again developed its  $V_1$  and H901 same

Craigie knew at the time that the two strains had been isolated during the same outbreak in 1918.

Another striking example of stability of  $V_1$  phage type is that relating to type T. This type was identified during a small typhoid outbreak in Britain in 1943. The carrier responsible for the outbreak

Outbreak of typhoid and it was soon found that type 1 bacilli were in 1944 still common in typhoid patients in Johannesburg and Pretoria.

appear that the test is positive in 9 out of 10 chronic carriers. When the carrier condition has lasted for a very long time, for example, periods of 30 or 40 or more years, the power of producing Vi antibody

served suspensions were adopted instead of living cultures (Félix, 1938) and especially since the introduction of Bhatnagar's strain ViI, which is a pure reagent for the demonstration of typhoid Vi agglutinins (Bhatnagar, Speechly, and Singh, 1938). In Britain, the technique of the test has been standardized by the use of a standard

The Vi test can now be used for the detection among convalescent typhoid patients of those who may pass into the chronic carrier stage and prove a potential menace to the community. The customary three or four negative examinations of the excreta are no guarantee that potential chronic carriers are not discharged from hospitals. Such carriers can be detected by two tests for Vi agglutinins, the first carried out on the eve of discharge from hospital, the second after an interval of about 3 months. A steady or rising Vi titre will arouse suspicion as to a possible chronic carrier condition, a decreasing Vi titre or a negative Vi reaction will indicate freedom from infection.

A scheme has now been adopted in Britain for the routine applica-

ice cream vendor, who was a urinary carrier. If the procedure now adopted had been in operation in 1938, when the carrier had his attack of typhoid fever, it is probable that the outbreak would not have occurred, since the ice cream vendor still had a significant Vi agglutination titre.

It is obvious that these new laboratory methods have put the epidemiological investigation of the enteric fevers on an entirely new basis. The public health authorities are now in a position to re-organize and intensify the campaign against the chronic carrier. It appears to be possible to devise a long term policy, based on close cooperation between clinician, epidemiologist, and laboratory worker, that might in time lead to the complete eradication of enteric infection.

area

It soon became clear, however, that there was need for standardizing the typing procedure in order to avoid faulty technique and consequent confusion. Extensive investigations of the various factors that determine the outcome of the typing tests were, therefore, carried

July 1947, and were adopted as the provisional international standard method. The Central Enteric Reference Laboratory in London acts as the international reference laboratory for enteric phage typing. Standard Vi phage preparations and the corresponding Vi-type strains are distributed to the National Reference Laboratories in various parts of the world, and the latter send to the International

uted in the same manner

#### DETECTION OF CHRONIC TYPHOID AND PARATYPHOID-B CARRIERS WITH THE AID OF Vi AGGLOUTINATION TESTS

The detection of the methods of isol greatly improved if excretion frequently is intermittent, so that repeated examination of the excreta over a long period of time may be necessary.

Most workers now employ the Vi agglutination test.

the reaction is independent of the intermittency in the excretion of bacilli. Numerous cases have been recorded of chronic carriers yielding negative faecal specimens over periods of many weeks or months. Yet the tests for Vi agglutination which were carried out during those negative intervals gave the same positive result on each occasion and thus enabled carriers to be detected who had previously been considered to be cured.

From my ex-

# THE CLASSIFICATION OF THE DYSENTERY BACILLI

BRIGADIER J S K BOYD, *Director, The Wellcome Laboratories of Tropical Medicine, London, England*

## INTRODUCTION

The first dysentery bacillus was discovered by Shiga in 1898, and numerous other related organisms have been described from time to time in the intervening 50 years. During the late war bacteriologists of the Allied armies, using the improved methods of isolation and identification made available in recent years, made exhaustive in-

neither common nor of great consequence. The time is, therefore, ripe to decide upon a scheme of classification which will meet with general agreement, and will be adopted universally in textbooks of bacteriology and other writings on this subject.

## SUBGROUPS DIFFERENTIATED BY BIOCHEMICAL REACTIONS—ADVANTAGES AND DISADVANTAGES

The classification within the group which has evolved in step with the discovery of new types of dysentery bacilli is based partly on biochemical characters (i.e., enzymic pattern) and partly on antigenic structure. Two main subgroups are distinguished by their power to ferment mannitol. This is a character of great constancy, to which, however, one or two clear cut exceptions are known. Further subgroups are made using indole formation as the index in the non-fermenters, and the fermentation of lactose in the fermenters. Within these groups a variety of other characters are used, some of which are of nomenclature but of much advantage if some general scheme is adopted.

A suggestion has recently been made (Weil, 1946) that the present

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## ABSTRACT OF DISCUSSION

Dr O FELSENFELD (United States) We have used the  $V_i$  agglutination test in more than 150 carriers and find the test extremely helpful in the carrier state. The new glycerinated antigens, which remain good for a long time, make it possible to perform

should like to ask Dr Felix in what percentage of persons, who are not carriers, the test will be positive

Dr A FELIX (United Kingdom) I was glad to hear Dr Felsenfeld's experience, and should like to know what strains he uses. Our strains contain small amounts of H and O antigens, but behave as though they were pure  $V_i$  antigen. The stability in saline seems to depend on the amount of  $V_i$  present. The standardization of these antigens can be made only by standard serums.

In reply to Dr Turner, approximately 5 percent of the normal population in Great Britain have suspicious agglutination tests. Because of this, any routine survey (e.g., of waterworks employees or food handlers) must be interpreted cautiously and investigated thoroughly.

(3) Whatever the taxonomist may decree, the biologist will continue to use the biochemical tests by which is subdivided as a routine step in the identification of an organism. These reactions are a valuable preliminary tests, and constitute useful confirmatory evidence of the strain. Thus, there is no cogent reason for abandoning classification from the standpoint of practical procedure. Contrary a sound argument for preserving a system in consonance with everyday routine.

It, therefore, appears desirable to retain, though with amendments and additions which are mentioned later, the scheme of the scheme at present followed.

#### ANTIGENIC STRUCTURE OF DYSENTERY BACILLI

*Shiga's bacillus*—Within the subgroups defined by tests, antigenic types can be recognized by serological

#### ANTIGENIC STRUCTURE OF CERTAIN FLEXNER GROUP ORGANISMS (BOYD)

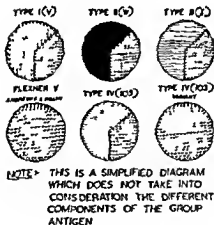
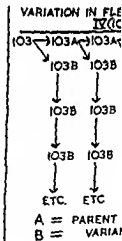


Figure 2



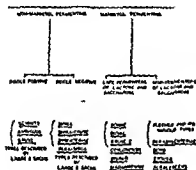
Figure

Others, and in particular complex antigenic pattern, and as there is still considerable subject, it is proposed to discuss it in some detail.

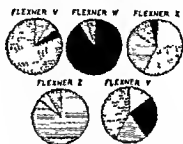
*The Flexner group*—The existence of a variety of

plemented by inserting the names now in common use between the generic name and the numeral (*Shigella Flexner* IV, *Shigella Shiga* XVI, etc.). It is claimed that either system would bring the classification of the dysentery organisms into line with that now adopted for the *Salmonella* group and would eliminate anomalies and lead to a general simplification.

Table 1.



ANTIGENIC STRUCTURE OF FLEXNER  
GROUP BACILLI  
(AFTER ANDRIEWES AND INMAN)



**Figure 1.**

Though the proposal has certain attractive points, there are also



and table 2). The variant antiserum can be completely absorbed either by the variant organism or by the original parent strain. From this, it is clear that the variation consists in the loss of an antigen which is present in the parent strain and is completely lacking in the variant. In other words, there is a complete antigenic modification.

A  
the  
serum of the parent strain (table 3).

These variants, and particularly the variant of Flexner IV, are very closely related to Andrewes' and Inman's Y strain. One type strain, Hiss-Russell Y (HRY), almost equals the group variant of Flexner IV in its power to absorb heterologous agglutinins from the antiserum of the parent strain of Flexner IV (table 4). Neither Flexner IV variant nor HRY completely cross absorbs the antiserum of the other, less than

monovalent serum, it is most probably a variant of W (table 5).

The X strain of Andrewes and Inman also occupies a controversial position. At one time (Boyd, 1938) I expressed the opinion that it

ABSORPTION OF FLEXNER TYPE II (005) ANTISERUM

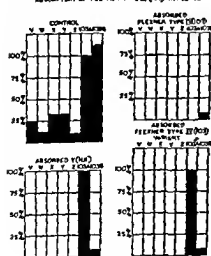


Figure 4.

Table 2.

ABSORPTION OF ANTISERUM OF  
FLEXNER TYPE II (005)

ANTISERUM	SUSPENSIONS						
	I (01)	II (02)	III (03)	IV (04)	V (05)	X (06)	Y
UNABSORBED	25	15	10	100	200	30	30
ABSORBED IV	—	—	—	—	5	—	—
ABSORBED V	—	—	—	100	5	—	—
ABSORBED Y	—	—	—	100	5	—	—

Table 3.

ABSORPTION OF ANTISERUM OF FLEXNER TYPE II (01)  
WITH TYPE I VARIANT

ANTISERUM	SUSPENSIONS						
	I (01)	II (02)	III (03)	IV (04)	V (05)	X (06)	Y
UNABSORBED	<1	1	7	12	100	30	20
ABSORBED	—	—	—	<1	100	—	<1

which they named V, W, X, Y, and Z (fig 1). They recognised 4 main antigens, of which dominating quantities of 1 and minor quantities of the other 3 were believed to occur in V, W, X, and Z, while Y possessed relatively equal quantities of either 3 or 4 of these. They

this monospecific serum is little less than that of its unabsorbed precursor. Such a serum will agglutinate the homologous organism, and the homologous organism only. Antisera with these characters have been prepared for all six types mentioned.

Although this hypothesis has found general acceptance it has

advantage be recalled.

The key to this problem is to be found in a study of the antigenic structure of variants which certain of these types throw off when

clumping to a significant percentage of its titre the other members of the Flexner group. After a variable time in artificial culture, variants are produced by this strain which give colonies of characteristic appearance on agar plates. These are larger, more irregular in outline and contour and usually more translucent than the parent colony. Apart from their woolly appearance, they have none of the other characters associated with true roughness. Unlike the parent strain, the variant is agglutinated to high titre by unabsorbed antisera prepared from the other Flexner types. The variant breeds true, while the parent strain continues to produce colonies of both types (fig 3).

This conclusion is based on the following findings

(1) X organisms completely absorb all group agglutinin from Z antiserum, leaving a monospecific serum which clumps only Z organisms. This absorption is accomplished with little or no loss of titre for the homologous organism (table 6)

(2) X antiserum is completely absorbed by Z organisms, leaving no agglutinins either for X or Z (table 7)

(3) X antiserum may be completely absorbed by VZ organisms (VZ being a strain rich in the Z type of group antigen). In some cases a residuum of Z agglutinin, or of equal quantities of X and Z, remains (table 8)

It is maintained that these results permit of only one interpretation, that X is a variant of Z.

In the last few weeks there has been an interesting confirmation of this conclusion. In 1935 the late Dr W M Scott sent a number of strains of dysentery bacilli to the national collection of type cultures one of which was type Z. A dried culture was prepared and in addition

was found to be a true Z, although containing a relatively large proportion of active bacilli.

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their occasional occurrence among strains submitted to reference laboratories for identification.

the specific antigen of Andrewes' and Inman's V (type 1) strain has types pro

Table 4

CROSS-ABSORPTION OF FLEISHER TYPE 100 (DONOR) AND YOUNG-RUSSELL									
ANTISERUM	SUSPENSIONS								
FLEISHER TYPE 100	100	100	100	100	100	100	100	100	100
UNABSORBED	5	30	100	50	25	100			
ABSORBED TYPE 100	—	—	—	—	—	—	—	—	—
ABSORBED TYPE 100	—	—	<	5	—	5	—	—	—
YOUNG-RUSSELL									
UNABSORBED	4	<	30	30	15	5	100		
ABSORBED TYPE 100	—	—	—	—	—	—	—	—	—
ABSORBED TYPE 100	—	—	<	—	—	—	—	—	—

Table 5

ABSORPTION OF FLEISHER TYPE 100 (DONOR) BY YOUNG-RUSSELL AND FLEISHER TYPE 100 (DONOR)									
ANTISERUM	SUSPENSIONS								
FLEISHER TYPE 100	100	100	100	100	100	100	100	100	100
UNABSORBED	5	100	4	25	5	10	100		
ABSORBED TYPE 100	4	40	—	<	<	5	5		
ABSORBED TYPE 100	<	15	—	—	—	—	—		
ABSORBED TYPE 100	5	30	—	—	—	—	—		

Table 6

ABSORPTION OF FLEISHER TYPE 100 (DONOR) BY FLEISHER TYPE 100 (DONOR) AND FLEISHER TYPE 100 (DONOR)									
ANTISERUM	SUSPENSIONS								
FLEISHER TYPE 100	100	100	100	100	100	100	100	100	100
UNABSORBED	5	100	4	25	5	10	100		
ABSORBED TYPE 100	4	40	—	<	<	5	5		
ABSORBED TYPE 100	<	15	—	—	—	—	—		
ABSORBED TYPE 100	5	30	—	—	—	—	—		

Table 7

ABSORPTION OF FLEISHER TYPE 100 (DONOR) BY FLEISHER TYPE 100 (DONOR) AND FLEISHER TYPE 100 (DONOR)									
ANTISERUM	SUSPENSIONS								
FLEISHER TYPE 100	100	100	100	100	100	100	100	100	100
UNABSORBED	5	100	4	25	5	10	100		
ABSORBED TYPE 100	4	40	—	<	<	5	5		
ABSORBED TYPE 100	<	15	—	—	—	—	—		
ABSORBED TYPE 100	5	30	—	—	—	—	—		

Table 8

ABSORPTION OF FLEISHER TYPE 100 (DONOR) BY FLEISHER TYPE 100 (DONOR) AND FLEISHER TYPE 100 (DONOR)									
ANTISERUM	SUSPENSIONS								
FLEISHER TYPE 100	100	100	100	100	100	100	100	100	100
UNABSORBED	5	100	4	25	5	10	100		
ABSORBED TYPE 100	4	40	—	<	<	5	5		
ABSORBED TYPE 100	<	15	—	—	—	—	—		
ABSORBED TYPE 100	5	30	—	—	—	—	—		

Table 9

ABSORPTION OF FLEISHER TYPE 100 (DONOR) BY FLEISHER TYPE 100 (DONOR) AND FLEISHER TYPE 100 (DONOR)									
ANTISERUM	SUSPENSIONS								
FLEISHER TYPE 100	100	100	100	100	100	100	100	100	100
UNABSORBED	5	100	4	25	5	10	100		
ABSORBED TYPE 100	4	40	—	<	<	5	5		
ABSORBED TYPE 100	<	15	—	—	—	—	—		
ABSORBED TYPE 100	5	30	—	—	—	—	—		

Table 10

BIOCHEMICAL REACTIONS OF FLEISHER TYPE 100 (DONOR) - NEWCASTLE - MANCHESTER									
	LACTOSE	GLUCOSE	MANNITOL	SUCROSE	SACCHAROSE	INOSITOL	GLYCEROL	GLYCERIN	GLYCERIN
FLEISHER TYPE 100 (DONOR)	—	ACID	ACID	—	—	—	—	—	—
FLEISHER TYPE 100 (DONOR)	—	ACID	ACID	ACID	—	—	—	—	—
MANCHESTER BACILLUS	—	ACID	ACID	ACID	—	—	—	—	—
NEWCASTLE BACILLUS	—	ACID	ACID	ACID	—	—	—	—	—
YET ANOTHER VARIANT	—	ACID	—	—	—	—	—	—	—

Table 11

BIOCHEMICAL REACTIONS OF FLEISHER TYPE 100 (DONOR) AND THE MANCHESTER VARIANT									
	LACTOSE	GLUCOSE	MANNITOL	SUCROSE	SACCHAROSE	INOSITOL	GLYCEROL	GLYCERIN	GLYCERIN
FLEISHER TYPE 100	—	ACID	ACID	—	—	—	—	—	—
MANCHESTER VARIANT	—	ACID	—	—	—	—	—	—	—



of V and W, the preparation of monospecific serum which in practice presents no difficulty, would be impossible. V antiserum would give

allied strains

Anomalous biochemical reactions are found in at least two of the

a nonmannitol fermenting type IV (table 11). There is general agreement that

(Ewing, 1946, Heller and Wilson, 1946, Lavington, 1946), and this appears to qualify for inclusion in the Boyd group

*Sonne's bacillus*—There is nothing new to be said about *Sonne's bacillus*

to one of the types in the Boyd Group

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## ABSTRACT OF DISCUSSION

Dr. S Mudd (United States). There are several appealing features in the dual classification. It is well known, however, that the enzyme-

variation in SA  
 rictions It was  
 a very familiar phenomenon, and appeared sometimes even on the  
 primary plate. It was agreed that group antigens were of very com-  
 plex structure, and the position was further complicated by the fact  
 that different rabbits gave a varying agglutinin response. While  
 differentiation into Wheeler's subtypes might be of some advantage,  
 it was felt that this was the thin end of a very large wedge. The  
 sion of the man-  
 as recommended  
 cable size

tract, is not a cause of dysentery. Nor have I found organisms of the dispair type, late fermenters of lactose and sucrose which lack

than in the dysentery group

Based on these conclusions, the following outline scheme of classification is suggested (approved types, within the headings given, will be serially numbered)

#### OUTLINE CLASSIFICATION

- (a) Late lactose and saccharose fermenters *Sh sonnei*
- (b) No late fermentation of lactose
  - (1) *Sh flexneri* I, II, III, etc (types with Flexner group antigen)
  - (2) *Sh boydii* I, II, III, etc (types without Flexner group antigen)

#### SUMMARY

Within these subgroups, types can be identified by antigenic structure, and given serial numbers

Valid types possess a distinctive antigen not found in any of the other types

Certain types share a second complex "group" antigen, and in artificial culture produce variants having only group antigen. Such variants, which include Andrewes' and Inman's X and Y strains, should not be classed as types

An outline classification is suggested



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## ABSTRACT OF DISCUSSION

Dr. S Mudd (United States) There are several appealing features in the dual classification. It is well known, however, that the enzyme content and the antigenic constitution are capable of independent variation. If classification depends on both being stable, what would hap-

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restrictions. It wa  
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e advantage  
wedge. The  
problem called for very careful consideration. Division of the man  
nitol fermenters into Shiga and Schmitz subgroups was recommended  
with the sole purpose of creating subgroups of manageable size

## COMPOSITION AND EFFICACY OF CHOLERA VACCINES

C G PANDIT, *King Institute of Preventive Medicine,  
Madras, India*

Since observations recorded in this paper are based mostly on Indian experience, it would be advisable at the outset to state briefly the nature of vibrios isolated from cases of clinical cholera in the country in recent years. A critical study of data available in this respect was made by Taylor (1941), who concluded that there was no evidence that any vibrios other than those of O group I were cholericogenic (Gardner and Venkatraman, 1935).

proportions in several outbreaks studied bacteriologically since 1934. Initially the greater preponderance of the Inaba type of vibrio and

northern districts entirely of the Inaba type. Contrary to the experience of the Japanese workers, there was no difference in the severity of infections between these types (Venkatraman and Pandit, 1938). It was considered at the time that this distribution more or less confirmed the hypothesis put forward on epidemiological grounds by Russell that the southern districts constituted an endemic area of cholera in the Madras Province. However, following a low incidence of cholera in 1939, a change in the prevalent type of vibrio was observed. During the period 1939-45 which included the widespread epidemic of 1942 and 1943, the Ogawa type of vibrio was almost exclusively prevalent throughout the Province. In 1947, however, again a year of low

siderable epidemiological interest and may have a bearing on the cyclical periodicity of epidemic cholera in India.

### COMPOSITION OF CHOLERA VACCINES

Cholera vaccines generally in use are prepared from vibrios belonging to O group I. As stipulated in India, the vaccine consists of a suspension of the vibrios obtained by washing off the growth from a 24-

hour agar culture with 0.85 percent saline solution. The vibrios are killed by the addition of 1.0 percent of phenol to the suspension without the application of heat, and the phenol content is reduced to 0.1 percent in the vaccine to be finally issued. The vaccine contains approximately 8,000 million organisms per milliliter. The usual public health practice in India is to administer the vaccine in a single dose of 1.0 cc.

fatal cases of cholera. It is customary in most laboratories to replace the strains used by new ones as they are isolated. Pending further information on the question of the virulence of vibrios, this procedure was considered to be the most suitable for adoption in the manufacture of cholera vaccines. However, it would seem that with the development of the technique for the measurement of antigenicity of vibrios

complete cross protection exists between the two sub types of cholera vibrios. In the Kan case, particular protection

Attempts have been made to manufacture cholera vaccines by growing the vibrio in a liquid medium. Linton and Jennings (1944), in their attempts to study the antigenic fractions of cholera vibrios, evolved a synthetic medium containing a minimum amount of nitrogen and a matter which favours a rapid multiplication of cholera vibrios.

medium for 3 days at 37° C, during which period the medium is supposed to become more or less completely exhausted. The growth is killed by the addition of 0.1 percent formalin and finally 0.1 percent of phenol. Mercuric nitrate is added as a preservative.

place during cultivation. By the use of the mouse inoculated protective test, the protective power of *V. cholerae* was found to be about 10 times greater than the protective power of the liquid vaccine.

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With regard to the distribution of the sub-types, the so-called Inaba and Ogawa types of vibrios, some of the recent findings are of considerable interest. These two types have been isolated in varying proportions in several outbreaks studied bacteriologically since 1934. Initially the greater preponderance of the Inaba type of vibrio and

origin of the strains. In one epidemic, the isolations in the southern districts of the Province were entirely of the Ogawa type and in the

the hypothesis put forward on epidemiological grounds by Russell that the southern districts constituted an endemic area of cholera in

and 1943, the Ogawa type of vibrio was almost exclusively prevalent throughout the Province. In 1917, however, again a year of low

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### COMPOSITION OF CHOLERA VACCINES

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In a critical study of the mouse protection test, Burrows (1947, p 261) has also emphasized the need to control the "major variables," i.e., the virulence of the challenge vibrio strain and the age and strain of mice to be used in the test, and to interpret the results with caution. The mouse protection test would then provide a definite basis to judge the antigenic potency of cholera vaccines. The technique as evolved by Sokhey has been accepted by the Cholera Advisory Committee in India as suitable for general adoption.

### REACTIONS FOLLOWING INOCULATION WITH CHOLERA VACCINES

Apart from the usual reactions associated with the use of bacterial vaccines, attention has to be drawn to the occurrence of a delayed reaction, especially after cholera vaccine inoculations. On or about the eighth day, the site of inoculation becomes red, swollen, and tender. This reaction is usually self-limiting and does not suggest definite

the protein, and the rate of development of antibodies in them are some of the factors which would explain the occurrence of the reaction with cholera vaccine particularly, and only in a certain percentage of the inoculated. The problem requires elucidation.

### EFFICACY OF CHOLERA VACCINE IN FIELD PROPHYLAXIS

Many attempts were made in the past to assess the value of prophylactic cholera vaccine inoculation in the prevention and control of cholera. The data recorded by several observers on the efficacy of cholera vaccines, e.g., the severity of the outbreak, the explosive character, the rapidity with which it reaches its peak, the phase of the epidemic in which mass inoculation campaign is instituted, and the homogeneity of the population at risk. It is not easy therefore to ascertain whether both the inoculated and the uninoculated populations were exposed to the same degree of infection. Besides, the existence of different strains of vibrios had not been sufficiently recognized.

recorded in each case. No significant difference in the antigenicity of the two vaccines was noted, both withstanding approximately 100 times more of the challenge culture than the unprotected mice.

#### STANDARDIZATION OF CHOLERA VACCINES

Various attempts were made in the past to devise suitable methods to measure the immunizing efficiency of cholera vaccine. Agglutinin response and bacteriolytic effect of sera of immunized animals were utilized in such studies. These are not discussed in detail since the question was reviewed recently by Burrows (1947). None of the methods advocated have come into practical use. In India, on the recommendation of the Cholera Advisory Committee, a general directive was given to the effect that the vaccine to be issued "should agglutinate with homologous test serum", and "should give protection to experimental animals against intraperitoneal infection with a homologous strain at a suitable level of test" (Taylor, 1941).

The fact that gastric mucin greatly enhances the virulence of many human pathogenic organisms for mice, and the subsequent observation of Griffiths (1942) that relatively small numbers of *V. cholerae* are required to set up a fatal infection when injected intraperitoneally in a 5 percent mucin suspension, led Sokhey (1944) to develop at the Haffkine Institute a method for a quantitative assay of the antigenicity of cholera vaccine.

to cholera vibrios, were used, though subsequently it was found that Swiss mice were even more susceptible. The uniformity in the virulence of test cultures was obtained by making fresh subcultures for

ance to a stated challenge dose is proportional to the quantity of the vaccine administered. The test thus aims to be more than a simple bio-

channels, tanks, and ponds, and these are used not only for drinking purposes but also for bathing and washing. The washing of infected clothing and other materials in or near such sources is not uncommon. The contamination of the different sources is, therefore, only a question.

and friends congregating at funerals and partaking of common meals thereafter is yet another contributory factor in the spread of the disease.

How the conditions enumerated above contribute to the general infestation of the population with *V. cholerae* in an epidemic was indicated by Venkatraman (1945). The observations were made in 1 village which had reported 9 attacks and 6 deaths from cholera. Personal investigation had revealed that there were more cases ending in recovery which had not been recorded. In an examination of stools of 293 healthy residents who had cooperated in this investigation of whom 150 had been previously inoculated and 143 had

In these circumstances, to limit the boundaries of an infected area to a house or street where cholera has occurred or to the locality within a specified distance would not only be to ignore epidemiological considerations, but to introduce considerable complications as well. The selection of the village or hamlet as a unit for study seems therefore most feasible.

was specially deputed to check independently all the data so that the true significance of any errors or omissions, inevitable in large field surveys of this kind, could be ascertained. It was found that such errors as were detected were of a minor nature and of no consequential importance.

Chandrasekar (1947) has shown that the use of the village as the basic unit in a further reassessment of results by selecting as the unit not a village but a part thereof, viz, a "cheri" which in conditions prevailing in South India is mostly inhabited by people of distinctly

available evidence was consistent with the view that a low grade immunity was produced by prophylactic inoculations with the vaccine.

For many years, cholera vaccine has been extensively used in India as a personal prophylaxis during cholera epidemics, and we are more inclined to the view so well put by Burrows that if cholera vaccine was completely valueless as a preventive of infection, sufficient evidence in that respect would be available by now. On the other hand, the experience of public health officials in India is decidedly in favour of the view that the vaccine induces a reasonable degree of immunity against cholera.

Further evidence in this respect has been adduced by Adiseshan, Pandit, and Venkatraman (1947) in a statistical analysis of data col-

and vaccine containing both the Inaba and Ogawa types of vibrios

1 103

In 627 villages, two or more outbreaks of cholera occurred during the epidemic. The inoculations done since the first outbreak were regarded as anticipatory inoculations and represented the total population protected prior to the occurrence of the second outbreak. The incidence rates per 1000 per year in these villages were as follows:

lated, the chances of subsequent outbreaks among them were greatly reduced.

From the evidence available, the authors came to the conclusion that the immunity first manifested itself on the fourth day after inoculation and reached an effective level after the eighth day. There was evidence to show that the immunity lasts for a minimum period of 6 months and

cessive days after inoculation.

From these studies, the over all conclusion was drawn that cholera inoculation afforded a definite degree of protection against an attack of cholera.

The validity of the foregoing conclusions obviously depends on the criteria adopted to define the population at risk and the care taken in the initial compilation of data. The authors had adopted the census



which have to be overcome to ensure conditions to provide data which would not only meet the requirements of a medical worker, but which would satisfy the demands of the statisticians

With the limited funds available at their disposal, Dr Pandit and his colleagues utilized the routine machinery of a Public Health Department to provide data of unusual accuracy which I think can reasonably stand the critical analysis of any epidemiologist. The attack rates in the inoculated and noninoculated groups in the first 3 days of inoculation are of comparable magnitude, dispelling all doubts once and for all of the comparability of the inoculated and noninoculated populations. The statistical units defining the population at risk was chosen taking into consideration the epidemiology of cholera in that area and the social and economic considerations of the community.

The data collected during the main enquiry lent themselves to further critical analysis, and statistical units to define prominently the basic attack rates in the inoculated and noninoculated populations in the different outbreaks showed a strong correlation, supporting satisfactorily the test made by Greenwood and Yule in 1915 for the accuracy of the data. It is a matter of satisfaction that, working under great practical difficulties brought about by the exigencies and severity of the cholera outbreak, data of such unusual accuracy could be obtained.

Dr H. A. REIMANN (United States). I am rather surprised that only one type of organism was found. I was in Chungking during the cholera epidemic there, and brought back several strains. Different sections. Are the cases Dr saw many cases in Chung

am). In cholera epidemics ty, confined to one single dose of vaccine, a procedure which was admittedly of limited efficiency. Has Dr Pandit experienced results of immunization with two or more doses of vaccine given at properly spaced intervals?

Dr A. FELIX (United Kingdom). We must make a distinction between endotoxin and exotoxin producers. It seems to me that we

more certainly does not inspire much confidence. The view

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light

seems somewhat more frequent in those over the age of 10 not used intradermal inoculations

lower social, economic, nutritional, and sanitary standards. Even so,

to the protection conferred on the individual during a period of 5 months after inoculation

greater degree of protection that even under such conditions is not likely to yield less protection than what has been found by the statistical analysis

In conclusion, it can be stated that cholera vaccine prepared from antigenically suitable strains has a definite place in any measures to be adopted in the prevention and control of cholera epidemics. Recent evidence furnished by Burrows (1917) based on his study of the experimental infection in guinea pigs, supports this view

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#### ABSTRACT OF DISCUSSION

Dr C CHANDRASEKHAR (India) Dr Pandit has referred briefly to the recent work in India on the assessment of the efficacy of anti-cholera inoculation in the field. Anyone who has attempted such field studies would be aware of the formidable practical difficulties

## THE ELECTRON MICROSCOPY OF MICROORGANISMS

Dr RALPH W G WYCKOFF, *Laboratory of Physical Biology  
National Institute of Health, Bethesda, Md*

The electron microscope has become an essential tool in the study of microorganisms because of the truly astonishing extension of vision it provides. Existing instruments have one hundred times the resolving power attainable by optical means and thus open up for observation those minutest of living forms that lie in the transitional zone between ordinary bacteria and the small viruses. The individual molecules of many normal constituents of living matter also are big enough to be easily visualized. The study already begun of the relation between them and the virus particles with which they are often associated is obviously only the first step in a new kind of inquiry into the molecular structure of both healthy and diseased protoplasm. In this paper elec

tion

The properties of electrons are such that those effective in present day microscopes cannot penetrate and delineate the internal structure of any but the thinnest of objects. Thus red cells are too thick for satisfactory examination, but a revealing insight can be gained into the structure of such objects as the gametocytes of malaria (fig 1) and the infecting agents (1) of the spirochetal diseases (fig 2). Present day electron microscopes will show only the silhouettes, capsules and flagellar systems of many bacteria, but there are others transparent enough so that details of their protoplasmic structure can be discerned. Thus globular molecules in the protoplasm of *E coli* are clearly apparent in figure 3. Particles of large viruses and rickettsiae (2), the

made familiar through the work of McFarlane and Anderson, and of Rake and his co workers and the noteworthy recent photographs of McFarlane. Psittacosis and feline pneumonitis particles (4) are spheres containing so little fluid that they collapse in a distinctive fashion during the desiccation required for electron microscopy (fig 4)

important. In our preliminary studies we found this not to be an important consideration. I have no doubt that two injections of vaccine are superior to one. In India, we are forced to rely on one, however. Nor can we give anticipatory inoculations. We must wait until cholera strikes an area before we know where best to concentrate our efforts.



Figure 5—Groups of mature particles of the T3 strain of colon bacteriophage which have begun to assume an ordered arrangement as they cluster together. A single mature particle with its spherical head and long slightly curved tail is at the left center. The tails on particles of this strain of bacteriophage appear only in shadowed preparations of the purified material. In some more closely packed groups of these particles the regularities of packing have been much more pronounced. Chromium shadowing. Magnification—21,000 X.



Figure 6—An electron micrograph showing the regular three-dimensional arrangement of particles composing a single crystal of one of the tobacco necrosis viruses. Palladium shadowing. Crystals from Dr. K. W. Smith and Dr. R. C. Markham. Magnification = 46,500 X.

Figure 7—A pair of colon bacilli whose protoplasm has been almost completely converted into developing bacteriophage particles as a result of infection with the T3 strain of this bacteriophage. Practically all these particles have collapsed heads, many of which carry a central granule. At the top fragments of the disrupted cellular membrane lie on the substrate and cover a small portion of the cell. Chromium shadowing. Magnification = 24,000 X.



Figure 8—A single colon bacillus after infection with the T3 strain of bacteriophage. The regular network formed by the developing bacteriophage and extending throughout the bacterial residue is apparent as a series of pits. Chromium shadowing. Magnification = 21,000 X.





Figure 1. An electron micrograph of a cyanobacterial cell showing the internal details. The cell is surrounded by a thick cell wall. Seen at higher magnification the protoplasm has a rather perceptible fine structure. The small particles distributed over the surrounding substrate are minute fragments of salt that were not thoroughly removed by washing. This preparation was stained with uranyl acetate and then exposed. It is needed to bring the internal details that shadows on the substrate are no longer apparent. These organisms were prepared through the courtesy of Dr. C. L. Atney. Magnification 15,000 $\times$ .

Figure 2. A thin film is lysed through the action of the Tetracycline. An internal protoplasmic part of the cell is pointed upwards as immediately at the left of the thick mass at the top of the cell. A few elongating particles are visible within the protoplasm. Where the protoplasm has been lysed the disrupted bacterium is only seen to consist of numerous glialular particles of very small size which are interspersed with the material. Chromium shadowing. Magnification 15,250 $\times$ .

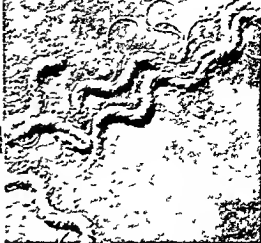
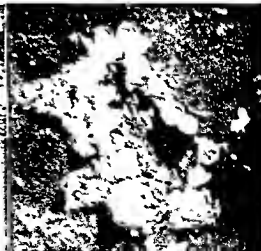


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Figure 3. A group of elementary bodies of putative cyanobacteria. The mature elementary bodies are the partially collapsed spheres which in this photograph have diameters of about 2 micrometers. At the bottom of the figure are three much larger objects which are out of focus which seem to be filled with similar but smaller spheres. There may be steps in the development of the elementary particles of this virus. Chromium shadowing. Magnification 14,000 $\times$ . This virus was provided by Dr. Norland Davis.





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## Session 5 LEPTOSPIROSIS, EFFECT OF ENVIRONMENT

Friday, May 14, 2 to 4 30 p m  
Departmental Auditorium, Room B

### LEPTOSPIROSIS IN JAVA AND SUMATRA

H ESSEVELD, M D, *Bacteriologist, Pathological Laboratory  
Medan, Sumatra*

Although the first clinical case of Weil's disease in Batavia was recognized as early as 1892 by Van de Scheer, it was by Vervoort's research in 1922 that it became clear that relatively mild leptospirosis is rather frequent among the laborers in several estates on the east coast of Sumatra. Thereafter, more information about this disease was laid down in many publications by Baermann, Kouwenaar, Wolff and others.

confined mostly to the men who work in the fields, and especially when the work is in connection with drainage systems, such as cleaning ditches and brooks.

As said before, the disease is usually relatively mild, Kouwenaar observed that only 10 percent of his patients had jaundice. The death rate, accordingly, is rather low. Typical hyperaemia of the conjunctivae, signs of nephritis, and muscular pains are very helpful in the diagnosis.

In routine work in the laboratory, blood samples from suspected cases are cultivated in Vervoort's medium, and agglutination lysis tests with a limited number of serological types are performed.

This brings us to the question, "What serological types can be distinguished?" At first Vervoort gave the name *L. pyrogenes* to all the isolated strains. However, in later years it appeared by improved methods of serological determination, e. g., the agglutination lysis test of Schuffner and Mochtar, that several types were involved, e. g., the Salnem and the Rachmat type (type strains isolated by Baermann in 1923).

Dr R. W. G. Wyckoff (United States) All of the spirochetes that

phage is a different and more complex mechanism

was also infected. In Macassar only a few *R. r. breviceaudatus* and many *R. concolor* were examined. There *R. concolor* carried *L. jaranica* in 9 percent. Of a total of 89 rats (*R. collieri*, *R. r. palembang* and *R. r. griseiventris*) caught in a small area in Kwala Begumit on the east coast of Sumatra, about 16 percent carried *L. jaranica*, whereas, this leptospira type could not be found in more than 1,000 rats from other areas on the east coast. Evidently *L. jaranica* can maintain itself in several field rats. *R. norvegicus* in the rural surroundings of Batavia was sometimes a carrier. A few carriers were present in *R. r. diardii*, a house rat, in Batavia and in Macassar. It must be stated, however, that *R. r. diardii* in rural or semirural districts can often be caught in the fields. The same is true for *R. palembang* a house rat, in Sumatra.

*L. jaranica* is nearly avirulent for guinea pigs and mice, although part of the animals, and especially the mice, became carriers after inoculation with these organisms. It is not yet certain that *L. jaranica* has any importance in pathologic conditions in man.

The majority of the 46 million people of Java work in rice fields which are flooded during long periods. *R. r. breviceaudatus* is extremely numerous in these fields, and it may safely be assumed that millions of people are exposed to infection with *L. jaranica*. Still not

determined) *Javanica* reactions were negative. Seroreactions of the relatives of these laborers, who were living near the area, were all negative. Therefore, it may be assumed that these 10 men were infected during their work. From this area 70 rats were caught a few weeks later. It was noted (table 2) that the rats were all small, thin, with fully determined) *Javanica* reactions were negative. Seroreactions of the relatives of these laborers, who were living near the area, were all negative. Therefore, it may be assumed that these 10 men were infected during their work. From this area 70 rats were caught a few weeks later. It was noted (table 2) that the rats were all small, thin, with fully

of the human infections on the east coast so occurs in Nusa Kambangan was

another more abundant animal reservoir of *L. jaranica* occasionally may become carriers.

tempt to classify serologically 60 leptospira strains of human origin showed that 20 percent could not be placed under the 5 mentioned types

Meanwhile, in Amsterdam five new types (Djasiman, Sarmin Sentot, Benjamin, and Naam) were determined by Walch Sorgdrager and Schuffner out of material previously collected in these parts of Sumatra by Wolff and Kotter. The frequency of these types in human

, the Japanese

In other parts of Sumatra, leptospirosis has occasionally been described, as in Bangkinang (1932, Slot and Van der Walle) where the Rachmat type could be distinguished, and in Benkulen (1931, Mulder et al.) where *L. icterohaemorrhagiae* was isolated and where also *L. bataviae* and *L. rachmat* infections were probable according to the seroreactions in patients.

In 1939 and 1940 Mochtar and De Reede succeeded, by a thorough

mat in 30 *L. icterohaemorrhagiae*, *L. solinem*, and *L. hebdomadis* were also present. There was serological evidence of 1 infection with *L. seje* and 1 with *L. ballico*. From 38 patients a leptospira culture

1/5000 after 1 year and 1/250 to 1/500 after 2 years in a total of 20 reactions. This is in agreement with the observations in Europe regarding infections with *L. icterohaemorrhagiae*. Older publications of Biermann and Wolff gave the impression that in the Indies the titers dropped under 1/10 within a year after the patients had recovered.

In Batavia a total of 100 sera were examined, 10 from patients with jaundice, 10 from patients with fever, 10 from patients with headache, 10 from patients with abdominal pain, 10 from patients with backache, 10 from patients with general malaise, 10 from patients with other symptoms.

*L. bataviae* infections and 1 *L. rachmat* infection. Most of the observed patients had jaundice and no less than 7 of 19 died of the infection. From other parts of Java, and also from Borneo, Celebes, Bangka, and Billiton a few cases of *L. bataviae* infections have occasionally been reported, usually as a disease with severe symptoms.

An examination of sera sent to the Fijkmann Institute in Batavia for the usual bacterial agglutination tests showed that in 673 sera from 617 persons, in 44 cases (7.1 percent) a positive reaction for *L. bataviae*

TABLE 3—*Leptospira* in various animals

Kind of animal	Location	Number	Leptospira cultures from kidneys		Leptospira types	Agglutination lysis reactions with sera of the same animals
			No	Pct		
Dog—	Sumatra, Niden	106	6	6	6 <i>L. hebdomadis</i>	
	Java, Batavia	132	8	6	3 <i>L. type II O</i> —	
	Celebes, Macassar	35	1	3	1 <i>L. bataviae</i>	
	Java, Batavia				1 <i>L. penicillata</i>	
	SIK O				1 <i>L. cellio</i>	
Cat —	SIK O	174	0			
	SIK O	213	13	6	7 <i>L. bataviae</i>	37 percent positive most <i>L. bataviae</i>
Bat ( <i>Cynopterus</i> )	Java, Batavia	400	2	—	1 <i>990</i>	<1 X O negative.
Squirrel		172	0		3 <i>220</i> —	12 K O 28 percent positive, 2 <i>bataviae</i> , or
Common rat		127	0			>2 K O 64 percent positive and 2 juveniles
Herpestes (carni)	Java, Batavia	7	1	—	1 <i>L. bataviae</i>	
Various carnivora		41	0			

The animal reservoirs of *L. rackmat* and *L. hebdomadis*, both occurring in Sumatra and Nusa Kambangan, have not yet been found in these islands. It may be stated here that *L. aliyami* A (closely related to *L. rackmat*) and *L. hebdomadis* in Japan maintain themselves in *Microtus montebelloi* (and *Apodemus speciosus*).

The results of investigation of other animals are compiled in table 3. Although dogs were found to carry leptospirae in Medan (6 percent, Kouwenaar and Wolff), in Batavia (3 percent), and in Macassar (Mochtar and Collier), no *L. canicola* was encountered in the 12 isolated strains. In Medan, *L. hebdomadis* and type "II C", in Batavia, *L. bataviae* and *L. pomona*, and in Macassar one strain of *L. ballico*.

*L. bataviae* and *L. javanica*, as is shown most clearly by the seroreactions of the adult animals, which are in more than 50 percent positive.

The dog must be regarded with suspicion as sources of leptospira infection of man.

Pigs in Batavia are frequently infected with *L. pomona* (Mochtar), and positive seroreactions in exposed persons indicate the considerable infectivity of this type for man.

From late (G. nagasaki) in Batavia a few leptospira were

or if they are merely a reflection of other unknown animal reservoirs is again an open question. The role of the bat as a source of infection in man does not seem important.

In conclusion, it may be stated that apart from the confusing number of leptospira types in man, the problem in these islands has in some respects become more complicated in this stage of research. For scientific purposes there is every reason to continue and to investigate

TABLE 3—*Leptospirae* in various animals

Kind of animal	Location	Number	Leptospira cultures from kidneys		Leptospira types	Agglutination lysis reactions with sera of the same animals
			No.	Pct.		
Dog—	Sumatra, Medan	106	6	—	14 <i>L. Achdamella</i> 12 <i>L. type II</i> O --- 3 <i>L. bataviae</i> 1 <i>L. pernix</i> --- 1 <i>L. bullocki</i> ---	37 percent positive most <i>L. bataviae</i>
Cat --	Java, Batavia Celebes Macassar Java, Batavia { < 15 K. O > 15 K. O	35 174 243	2 1 0 12	— — — 3.9	7 <i>L. bataviae</i> (1 "OC 12 "OC	(1 K. O negative, 12 K. O 53 percent positive, <i>L. bataviae</i> or > 2 K. O 64 percent positive and <i>L. javanica</i> .
Bat ( <i>Cynopterus</i> )— Squirrel Coconut rat Herpestes (arm.) Various carnivora	Java Batavia Java Batavia, ---	400 172 177 7 41	3 0 0 1 0	— — — 100 —	1 <i>L. javanica</i>	

## DIAGNOSIS AND TREATMENT OF LEPTOSPIROSIS

P. H. VAN THIEL, *Institute of Tropical Medicine, Leiden, Netherlands*

It seems desirable to confine myself to a few essentials and to a discussion of those points where no agreement exists and which might influence our future action and scientific research.

### DIAGNOSIS

A completely developed case of leptospirosis cannot be mistaken

tory. In cases of influenza, special difficulties may arise, as it begins, in the same way. Here the eyes show a catarrhal affection, in leptospirosis a pericorneal injection of the blood vessels of conjunctivae frequently exists.

Sheldon (1945) suggested that biopsy of the calf muscle may be a useful measure in diagnosis of Weil's disease. The earliest lesion consists in the appearance of small and medium sized vacuoles in the cytoplasm of the striated muscle fiber. This tends to become confluent, and simultaneously the cytoplasm of the fiber in the involved area loses its cellular detail. The lesions are repaired from about the seventeenth day onwards. In more severe lesions repair is accompanied by fibrosis. The lesions always involve a part only of one muscle fiber, sometimes two or three adjoining fibers show focal involvement. It is necessary to determine whether these changes occur in other leptospiroses as well and if this biopsy has any practical value.

It being necessary in severe cases of leptospirosis to treat the patient specifically or otherwise, it is of the greatest importance to make an

trifuge blood liquid mixture for 15 minutes with a 1,500 speed if necessary the plasma for 30 minutes with a 3,500 speed)

possible to isolate the causative organism. This might also be done with the cultural test.



tion of the tubes is not delayed for longer than some 16 hours after the onset of the test, one often prevents, in the strongest dilutions above the limit of the lysis, the presence of the structures named by Bessemans (1940), "agglutinats leptospiriens terminaux." About their nature no uniformity of opinion exists.

We recommend the performance of these tests with the strains of those types of leptospira native to the country and with others that might be expected to be found there, although this is made more and more difficult by the diversity of isolated strains, especially in the tropics.

In the Netherlands a reaction is considered to be positive when the titre of 1:300 is reached, but in Indonesia, and perhaps in other tropical regions as well, it is recommendable to increase this titre to 1:3000 as a titre of 1:2500 is frequently met among healthy persons (Mochtar and de Reede in Noesakambangan, 1941) and as Postmus (1934) observed that the titre of 1:2500 may exist for many years. More data

this period may amount in Weil's disease to more than 20 years. we described a patient of whom the titre of the serum was 1:50,000 in Europe, but which had decreased to 1:100 within 1½ years after his arrival in Indonesia. In leptospirosis febrilis the sera showed a

ed by Paetz  
ard (1942)  
n produces  
l is reliable  
Although  
it does not

seem probable at present that it will ever replace the microscopic tests owing to the fact that too much leptospiral culture is required for the performing of that reaction and because it is not possible to establish low titres of the sera under examination with it.

In countries where only one form  
plement fixation test, elaborated by  
Gachtgens (1933), may render good  
Dornick (1936) rightly considered it an unmistakable advantage

method for small laboratories, where the serological diagnosis of Weil's disease is carried out only at wide intervals because the antigen can be kept in stock for even 7 months. For tropical countries however the use of this test should be which is greater advantage it is less

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It seems desirable to confine myself to a few essentials and to a discussion of those points where no agreement exists and which might influence our future action and scientific research

## DIAGNOSIS

A completely developed case of leptospirosis cannot be mistaken. In mild, incompletely developed cases the clinical diagnosis remains only a supposition. This may be supported by the epidemiological argument. In such cases it is necessary to call in the aid of the laboratory. In cases of influenza, special difficulties may arise, as it begins in the same way. Here the eyes show a catarrhal affection, in leptospirosis a pericorneal injection of the blood vessels of conjunctivae frequently exists.

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with the binominal nomenclature as a species of *Leptospira*. When chiefly the carrier and serological characteristics of these separate species have become known, only then will it be possible to classify them. It is quite possible that this classification of groups will lead to the distinction of true species.

We do not object to the name transitional or intermediary form

same specimens of serum of Weil's disease patients, when examined immediately and again between 4 and 40 days after the first examination, that the reaction showed a titre in the second examination 8 to 16 times higher than in the first one. Afterwards this increased agglutinability dropped again. He explains this variability as due to the culture medium, in which normal rabbit serum is a varying factor. On this account it is not allowable to draw conclusions from small differences in the height of the titre in connection with the

exist between separate types.

Further, neither the animal test nor the geographical distribution sharply differentiates the different leptospiroses.

In the tropics the separation between the leptospiroses is much less sharp than, for example, in Europe. Here all actual and potential carriers of the different types must be known in connection with the serological relationship before there can be any talk of a definite classification.

Till now we have considered the delicate coiled filament with its hooked ends as the only form of appearance of the leptospira. Gas

tions by Séguin in other genera of spirochaetes (these *Blas* arise 1946) *ys* in after human e with 10 and ent to 18.

sensitive than this test at the moment that antibodies begin forming. As the character of the thrombocytobarin test must be called capri-

phenomenon interferes with the agglutination of the leptospirae, which takes place at the same time. A modified method of Bau Kien-Hun (1937) is recommended, using small tubes instead of slides.

For diagnostic purposes the Pfeiffer test is no longer applicable, mainly because of its constant requirement of very virulent leptospiral cultures at one's disposal.

The coagulation test by Carlinfanta (1935) does not come into consideration, as it is too complicated and as strain specific differences between varying types of leptospirae cannot be demonstrated.

Examination of the cerebrospinal fluid has no definite value for diagnosis. Leptospirae are probably not found longer in the fluid than in the blood. The agglutination lysis test may be performed with the fluid, but the titre seldom rises more than 1:100, and it is necessary not to attach too much importance to a negative result of the agglutination lysis test.

Now and then leptospirae appear in the urine. Agglutinins (the phenomenon of lysis can be observed as well) have been found in it by Van der Hooft (1925) for the first time. In the whole of the

As to convalescence, from a scientific point of view it is important to seek during a relapse for the presence of leptospirae in the peripheral blood, as it ought to be proved whether the relapse is caused by the renewed swarming of leptospirae into the blood.

What now is the value of the saturation test? At present it is the

to all pathogenic leptospirae, more characteristic of the species than the too sensitive agglutinable antigens. If this point of view is ac-

that this analysis, performed by authors who take the pluralistic point of view, is considered as the creation of undesirable complications.

In our mitigated pluralistic point of view, taken principally on practical grounds, we recommend denominating a freshly isolated strain which is not identical with other known strains provisionally

tination-lysis. According to our opinion, a few facts support proposition: Firstly, Yang and Theiler (1930) and Smith (1931) demonstrated with their cross-immunity tests that vaccination heterologous strains may also protect against an infection. Secondly, Kotter (1939) described an immediate and complete effect in a infected with the strain Sarmin, after the injection of fresh sera originating from the same strain.

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that reason we at present hesitate to consent to the use of heterologous sera for therapeutic purposes.

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former experience

Chemotherapy has not made great progress in recent years. Up to the present no satisfactory results have been obtained with sulphonamide preparations, neither in experimental leptospirosis nor in *in vitro* performed experiments. In recent years hexamine has been recommended, and sodium tartrobismuthate has given good results (Manson-Bahr, 1945).

of relapses; also, there was a general impression that the treatment of patients dramatically improved within 36 hours. On the other hand,

trial to give the treatment early, and the dosage should be large. We gave penicillin by slow intravenous drip to his patient when his condition after the administration of antiserum remained serious with

The patient gradually recovered with the experimental treatment. The patient, and McAllister's observations, penicillin had a suppressive effect on the disease.

## TREATMENT

It is allowable to consider the therapy of all leptospiroses from the same unicistic point of view, as the pathogenesis and the clinical features form a unity, although a few symptoms seem to occur more frequently (at least locally) in one form of leptospirosis than in another (e g, meningitis in leptospirosis pomona, swineherd's disease in Switzerland)

As to general treatment, no new points of view can be brought to the fore. An exception will be made in connection with the research of Kastein and Haex (1930) on the important disturbances of the blood supply of the parenchymatous organs. They found in the brain, liver, and kidneys diffusely scattered foci with a strongly reduced blood supply, complete parts contained almost no erythrocytes. They were also able to establish pathological changes in the ganglion cells of the brain, which must be attributed to ischaemia or to anaemia, and thrombi of polynuclear leucocytes in numerous small blood vessels in the brain and in the kidneys. It is impossible to say whether these disturbances are an immediate consequence of the intoxication of the wall of the vessels, or the nerves of the vessels are intoxicated and the reflexes of the vessels thereby disturbed. However that may be, therapy should take into consideration that these divergences in the blood supply and disturbances in the function of the named organs do occur.

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For that reason it is necessary to have the blood examined for the presence of leptospirae in severe cases of fever of unknown origin. When the icterus has completely developed and anuria has already come into being one can no longer rely on a successful recovery. In such cases Schuffner (1932) advises that one must repeatedly try the injection of large doses.

In a series of experiments it was found that the injection of large doses of one of the homologous strains acts most effectively.

with the aid of the homologous strain acts most effectively. Van Riel (1946), however, considers it necessary that the choice of the antisera for therapeutic purposes rests on the immunobiological characters of the diverse leptospiral types, viz on cross immunity and not on agglu-

## DEPOSITOS ANIMALES DE LEPTOSPIROSIS HUMANA

FERNANDO SAVINO Y EDUARDO RENNELLIA, Instituto Pacteriologico Malbran, Secretaria Salud Publica de la Nacion, Buenos Aires Republica Argentina

La leptospirosis humana pertenece al grupo de la zoonosis. Por tanto, trata-se de una enfermedad de los animales transmisible al hombre.

El agente etiológico, la *Leptospira*, tiene la característica de parasitar al riñon de los mamíferos que son sus depósitos naturales y de allí son eliminados por la orina al medio ambiente exterior.

En el riñon, la *Leptospira* forma verdaderas colonias aprovechando las tortuosidades de la parte distal de las paredes de los "tubuli contorti". Luego, atraviesan las paredes de los mencionados tubos y son arrastrados por la corriente urinaria hacia el exterior (Kwee Tat Tjhong, 1940).

La *Leptospira*, además del riñon, tiene cierta predilección por localizarse en el cerebro de los animales atacados. Así lo corroboran los trabajos experimentales de Kastein y Haex (1939) y el aislamiento de la *L. schuffneri* (Collier y Mochtar, 1939) del cerebro de quiropteros.

Una vez en el medio ambiente exterior, la *Leptospira* pueden vivir como saprofitos en el agua o en el barro un tiempo mas o menos largo. El lapso de tiempo depende del pH y de la concentración salina del agua. Desde allí, introducidas por vía bucal pueden infectar al hombre o nuevamente a los animales.

Sin embargo, en otros casos, el hombre o los animales pueden adquirir la enfermedad por contacto directo con los animales atacados por *Leptospira*.

Algunos animales, como la rata gris infectada con *Leptospira* (Savino y Rennellia, 1945) o con *L. bona*

tiempo de los citados microorganismos (Savino y Rennellia, 1945). Y la *L. grippo typhosa* no persiste más de un mes en el riñon del *Microtus arvalis* (Schuffner y Bolander, 1943).

Los animales espontaneamente infectados por *Leptospira* de preferencia son depositos naturales de una sola especie. Sin embargo una determinada especie en algunos casos, llega a parasitar a diferentes animales.

El estudio de los animales depositos naturales de *Leptospira* lo haremos de acuerdo al orden a que pertenecen los mamíferos.

I Orden Quiroptera (murciélago)

(1944) observed that even in concentrations up to 240 Oxford units  
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equally effective

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ence has however not been sufficient to enable us to judge whether penicillin therapy is superior to serotherapy and whether penicillin should be administered in order to sustain serotherapy. It should certainly be administered when no serum is available or in order to shorten the course of a relatively mild case of leptospirosis.

According to Heilmann (1945) penicillin is more effective than streptomycin but the latter may be a useful adjunct to penicillin.



Batavia, infección natural por *L. mitis* (*bataviae*) y por *L. pomona*.

Esseveld, Collier y Mochtar (1940) identificaron en perros de Sumatra, a la *Leptospira autumnalis* y a la *L. hebdomadis*.

Nordstrom (1941) en perros de Sucria reconoce infección espontánea por *Leptospira icterohaemorrhagiae*.

Kathe (1943) identifica en perros de Breslau (Alemania), la *L. icterohaemorrhagiae* y la *L. grippis typhosa*.

Finalmente, muchos son los autores que han descrito en el hombre la infección por *Leptospira canicola*. Citaremos entre ellos a los siguientes: Dhoot, Klarenbeek, Schuffner, y Voet (1934), Petersen y Jacobsen (1937), Schuffner y Walch, Sorgdrager (1937), Bramer, Petersen y Thompson (1938), Tetzner (1938), Meyer, Anderson y Eddie (1939), Savino y Rennella (1945), etc.

(b) Familia Felidae—Sub familia Felinae

*Felis domestica*—La investigación de *Leptospira* en gatos, fue realizada en Java por Esseveld y Collier (1938) y por Esseveld, Collier y Mochtar (1939-40).

Los estudios de los mencionados autores demostraron la infección espontánea de gatos, originada por la *L. mitis* y por la *L. javanica*.

### III Orden Rodentia

(a) Familia Muridae

(1) *Rattus norvegicus*, *R. rattus* y *R. alexandrinus*—La rata gris generalmente está parasitada por la *Leptospira icterohaemorrhagiae*. Otra especie de *Leptospira*, origina en el hombre la clásica enfermedad de Weil.

En el caso de *R. norvegicus* y *R. rattus*, la infección por *L. icterohaemorrhagiae* es espontánea.

Sin embargo, la rata gris también puede estar espontáneamente infectada por otras especies de *Leptospira*. Tan es así, que Mochtar y Collier (1939) y Mochtar y Esseveld (1939) aislaron *Leptospira mitis* de *R. norvegicus* y *R. rattus*.

En el caso de *R. alexandrinus*, Mochtar y Esseveld (1939) demostraron la infección espontánea por *Leptospira javanica*.

y por *L. mitis*.

Savino y Rennella (1945) describen a la *Leptospira bonariensis* en el caso de *R. norvegicus* y *R. rattus*.

En el caso de *R. alexandrinus*, Mochtar y Esseveld (1939) demostraron la infección espontánea por *Leptospira javanica*.

En el caso de *R. norvegicus* y *R. rattus*, Mochtar y Esseveld (1939) demostraron la infección espontánea por *Leptospira mitis*.

(3) *Rattus rattus brevicaudatus*—En las Indias Holandesas, se aisló de dicho roedor, a la *L. javanica*. Así lo demostraron los trabajadores de dicho país, Sardi to Mochtar, Wirasmo (1937) y Mochtar y Esseveld. Collier y Mochtar (1939) demostraron la infección espontánea por *Leptospira javanica* y *L. mitis*.

Collier (1940), estudiaron al

Collier y Esseveld (1938), en las Indias Holandesas, aíslan del "90c" Col que se trata

La citada especie fué aislada en Andamans, en un caso de leptospirosis humana (Das Gupta, 1941)

Mochtar y Mertens (1938), aislaron del cultivo "90c", otras tres cepas que denominaron "90cI", "90cII" y "90cIII"

Mochtar y Collier (1939), por cultivo de riñón de murciélago *Cynopterus*, obtienen las cepas de *Leptospira* "C3583" y "C3868" Ambas fueron reconocidas por Collier y Mochtar (1939) como una nueva especie y la denominaron *L. cynopteri*

También es interesante hacer notar que Rizzotti (1939) estudio en Etiopia, 71 casos de enfermedad de Weil, probablemente originados por quirópteros

## II Orden Carnivora

### (a) Familia Canidae

*Canis familiaris* —Klarenbeek (1927) en perros jóvenes de Utrecht, estudió una enfermedad aguda y mortal, caracterizada por ictericia, hemorragia y vomitos. En los cortes del riñón observó un micro organismo que denominó *Spirochaeta ictero-uraemiae canis*

Klarenbeek y Schuffner (1933) describieron a la *Leptospira canicola*

(Yugoeslavia) una enfermedad caracterizada por gastroenteritis hemorrágica. En los cortes de riñón de dichos animales, descubrieron el agente causal y lo designaron *Spirochaeta melanogenes canis*. También Okell, Dalling y Pugh (1925) describieron una *Leptospira* en el riñón de perros atacados de ictericia infecciosa.

La *Leptospira* en perros fué estudiada en diferentes países por los

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en Pensylvania (U S A) En Illinois, fué investigada por una comisión encargada del estudio de la leptospirosis animal (1943), Fraga de Acevedo (1943) en Lisboa, Gardner (1943) en Inglaterra, Perez Figueroa (1943) en la Habana, Savino y Rennella (1943) en Buenos Aires, etc

En perros, además de la *L. canicola*, han sido aisladas otras especies de *Leptospira*. Mochtar y Collier (1939) demostraron en perros de



citado roedor en Makassar (Indias Holandesas) y determinaron su infección por la *L. javanica*

(b) Familia Cricetidae

(1) *Apodemus speciosus*—Segun Aoki, Kaneko y Morimoto (1935) y Kaneko, Kotorn y Aoki (1935) en el Japón, dicho roedor es el deposito natural de la *Leptospira autumnalis*. Como es conocido, el citado microorganismo es el agente etiológico del 'Hasamiyami' o fiebre otoñal

(2) *Microtus montebelloi*—Kaneko, Kotorn y Aoki (1935) aislaron en dicho roedor a la *L. autumnalis*. Tambien el *Microtus montebelloi*, es deposito natural de la *L. hebdomadis* (Ido, Ito, Wani, 1918)

(3) *Microtus arvalis arvalis*—En Europa, es el deposito natural de la *L. grippotyphosa* y de la *L. sejeae*. Asi lo demostraron los trabajos de Rimpau (1942-43-45) y de Uhlenhuth (1943)

(4) *Apodemus sylvaticus*—Rimpau (1942-43-45) en Baviera aislo

*typhosa* y *L. sejeae*

(5) *Eutamias glareolus*—Uhlenhuth (1943) demostro en el mencionado roedor infeccion natural por *L. grippotyphosa*

(6) *Microtus agrestis*—Rimpau (1942-43-45) aislo de dicho animal a la *L. grippotyphosa*

(7) *Micromys minutus soricinus*—Mino (1941-42) estudio y aislo en los mismos en el Norte de Italia, la *L. mitis* y la *L. sejeae*

(c) Familia Capromyidae

(1) *Myocastor coypus*—Anchezar e Illa (1947) (trabajo no publicado) aislaron *L. bonariensis* en nutrias del Jardin Zoologico de la ciudad de Buenos Aires. Posiblemente la rata gris fue el origen de dicha infeccion

#### IV Orden Artiodactyla

(1) *Sus scrofa*—Johnson (1939), en Australia, observo que el cerdo es deposito natural de *L. pomona*

Terskikh (1940) en Rusia, aislo del cerdo una *Leptospira*, agente causal de infeccion humana y la denominó *L. del agua tipo II*

Johnson (1943), en South Queensland Australia reconocio en el cerdo la presencia de *L. pomona* y *L. mitis*. Ademas describio casos humanos de leptospirosis por contacto con porcinos infectados por las

*L. suis* y *L. hyos*. Dichos autores, tambien demostraron la infeccion por *L. suis* en casos de leptospirosis humana originados por cerdos y bovinos

Gsell (1946) observo en Suiza, infeccion en el ganado porcino, debida a la *L. pomona*.

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So there is no essential difference between the clinical pictures of the various leptospira infections in man. There are other examples of diseases with different etiology and the same clinical features, for instance, typhoid and paratyphoid B. So this is no argument for the unitarian theory. The virulence for experimental animals may differ too, but this is also more in degree than essential.

The epidemiology of several types, however, is quite different. Most leptospira types have a definite parasite host relationship. Several types have been found exclusively in rodents. Some are pathogenic for man, others thus far have not been recognized as such. In still other types only larger animals have been found to be carriers and responsible for the spread of the disease (*L. canicola*, dogs, *L. pomona*, hogs). The difference in epidemiological behaviour of *L. canicola* and *L. icterohaemorrhagiae*, which serologically are closely related is quite striking. *L. canicola* is found only in dogs who, after having suffered from the disease, may excrete the leptospires for a long time.

*L. icterohaemorrhagiae* is spread mainly by the sewer rat, which when infected remains a carrier for the rest of its life. Tame white rats used as experimental animals sometimes harbour these leptospires in their kidneys and excrete them in large quantities. Arvicola has been found excreting them with the urine. Dogs may become infected and sometimes have positive urine for a short time. So the circumstances under which *L. canicola* and *L. icterohaemorrhagiae* are spread are different.

Canicola infections in man depend on the contact with infected dogs mainly male animals. In Amsterdam most of the cases could be traced to dogs, often puppies, which excreted large numbers of *L. canicola*, sometimes several members in one family were infected. In some families the dogs were not available for examination, but only in a few was there no history of contact with dogs. A curious feature is that the 50 canicola infections observed during the last 15 years accumulated in the second half of the year, as is shown in table 2. In this period there must be special circumstances which favor the spread of the disease. Till now we have not found a reasonable explanation.

TABLE 2 — *L. canicola* infections diagnosed in the Tropical Institute Amsterdam

Year	Quarter				Total	Year	Quarter				Total
	First	Second	Third	Fourth			First	Second	Third	Fourth	
1933	0	0	2	0	2	1941	0	0	1	2	3
1934	0	2	3	0	5	1942	0	0	1	1	2
1935	0	0	0	0	0	1943	0	0	0	1	1
1936	0	0	2	0	2	1944	0	0	1	2	3
1937	0	0	1	3	4	1945	0	0	1	1	2
1938	0	0	1	1	2	1946	0	0	5	5	10
1939	0	0	0	0	0	1947	0	0	0	4	4
1940	0	0	0	1	1						
	0	2	0	5	15		0	0	15	19	34

# SIGNIFICANCE OF IMMUNOLOGICAL DIFFERENCES IN LEPTOSPIRAS IN THE DIAGNOSIS AND EPIDEMIOLOGY OF HUMAN LEPTOSPIROSIS

A. CHARLOTTE RUYE, M. D., J. E. MINKENHOF, M. D., AND J. W. WOLFF, M. D., *Department of Tropical Hygiene of the Royal Institute for the Indies and the Municipal Public Health Laboratories, Amsterdam, The Netherlands*

leptospirosis research in 1924 and continued with many coworkers till September 1944. After Schuffner left, the laboratory had to be evacuated, and in the period which followed, without gas and electricity, the collection was threatened with total destruction. Having been one of Schuffner's early coworkers, I took it over to my own laboratory, and with the help of the staff we managed to save it. After the liberation, the collection was transferred again to the Tropical Institute, and it is now under the direction of Dr. Wolff.

Schuffner (1) always stressed the fact that the various serological types of leptospires have to be regarded as biological entities. It is difficult to decide whether they are different species or merely types

third or even one tenth of the titer. Strains which are related some times are lysed to one third or even to the titer (*L. canicola*, *L. ictero haemorrhagiae*) of the other type. It is, therefore, necessary to test each strain with as many sera as possible (cf. Walch, Sorgdrager and Rebland, 1933, 1942).

when other sera give strong coreactions, cross agglutination and ab



A positive agglutination lysis reaction in the blood of field mice is only an indication that the animal has suffered from the disease not that it is really excreting leptospiras. In rats infected with *L. icterohaemorrhagiae*, however, the serological reactions often become negative, but the infection of the kidneys persists. Reliable information about the infectivity of these rodents can only be obtained by examination of urine or kidneys.

Another example of the close parasite host relationship is the swineherd's disease in Switzerland, recognized as an infection with *L. pomona*. All cases of the disease could be traced to contact with swine. In Switzerland many swineherds are infected with *L. pomona* in the Netherlands, this infection, as is shown by lysis reactions in swine sera in man either, despite the leptospirosis have been performed with *L. pomona* strains, too since 1912. In Indonesia a great deal of work has already been done to classify the leptospiras and study their epidemiological behaviour.

ology of the various types has been made, it is not admissible to classify them as variants of one species. In our opinion the main types are to be considered as different species. Complete and incomplete biotypes are known. It may be that there exist other minor differences, especially in absorption tests, which are too small to justify splitting off another species, but further study is necessary to enable us to make a reliable classification, especially for the types found in the tropics.

I should like to add a last observation made on myself. A few months ago I suffered from a leptospirosis hitherto unknown in man. I got the infection from a mouse spontaneously infected with *L. ballum*. Our whole mouse colony and that of one of the large breeders in Amsterdam proved to be infected with this strain, which thus far had been found only once by Schuffner in 1942 in a white mouse, and later by Borg Petersen in Denmark in a wild mouse.

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In the Netherlands, however, the classical Weil's disease is mainly an infection caused by water contaminated with urine of sewer rats which are infected to a large percentage. In our country there is a peak in summer and the beginning of autumn caused by swimming in rivers, lakes, and canals. War conditions are reflected in Weil's disease in Amsterdam because the blackout caused so many water accidents in the rat infested canals. In those years, the peak was no longer in the summer but in the winter months. When in January 1945 the curfew began at 7 o'clock, there were no more cases of Weil's disease. The absence of cases in the summer of 1944 is probably due to the high brackishness of the water caused by the overflow of salt water let in by the Germans.

TABLE 3—Cases of Weil's disease in Amsterdam

Year	Quarter				Total	Water accidents
	First	Second	Third	Fourth		
1935	0	0	4	0	4	1
1936	0	0	4	2	6	1
1937	0	6	10	0	16	4
1938	1	2	7	1	11	2
1939	0	0	7	8	15	7
Total	1	8	27	11	47	
1940	1	2	7	6	16	0
1941	4	2	6	10	22	17
1942	4	0	8	7	19	10
1943	3	0	3	0	11	7
1944	2	0	0	0	2	0
1945	0	1	7	2	10	2
Total	14	5	23	25	67	36
1945	0	2	8	8	18	8
1947	0	2	6	1	11	1

On the other hand, in the Netherlands, infections with *L. grippo*

bites were the portal of entry for the leptospiras from the infected urine. Large epidemics in flooded areas, such as have occurred in Russia, Germany, Switzerland, have never been observed in our country. Sporadic cases have been found in farm laborers in territories where the field mice were infected in a large percentage. The infection index, however, is not stable, the field mice population being one year infected nearly to a hundred percent and the next year not at all.

Schuffner (7) demonstrated that the kidneys of the infected mice contain thick layers of leptospiras in the tubuli. However, unlike the process in rats, this infection is not durable, and field mice which survived several months in captivity lost the leptospiras within a few months.

in France. Cases of Weil's disease were suspected but none were demonstrated by laboratory examination and these results were subsequently checked at the Pasteur Institute. In regard to the importance of hemoglobin to the growth of leptospira, my experience in a study of canine leptospirosis in Pennsylvania in 1939-40 showed that growth was materially enhanced when hemoglobin was added to the rabbit serum used in Schuffner and Mochter medium.

Dr H. ESSEVELD (Sumatra). I am very much impressed by the South American work in this field. Much of it has not been known to the Dutch workers. Dr Savino has suggested that the number of names be reduced. I should like to drop *L. mitis* which is identical with *L. bataviae* described 10 years previously.

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# ABSTRACT OF DISCUSSION OF PAPERS BY ESSEVELD, VAN THIEL, SAVINO, AND RUYS

DR K F MEYER (United States) There are many interesting observations in these papers. It is constantly attended by difficulty often is not prepared infrequently confused the preparation of anti-

and cannot be used

There are species specific strains of leptospira, and I was greatly interested in Dr Ruys' findings. Only with immune sera can we make the definitive differentiation. As to therapy, I agree that serum is excellent if and when available. We have tried penicillin and are convinced that it is efficacious.

Leptospirosis in dogs is an important problem. I wonder whether Dr Ruys' finding that the disease is more frequent during the last quarter of the year is true.

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DR E SAVINO (Argentina) I should like to add a few remarks regarding the diagnosis of leptospirosis in animals and man. We have utilized a saline suspension of the bacteria as an activating agent. Here we also add casein hydrolysate to the medium. It is possible that

the kidneys are aspirated for the inoculum. With *Leptospira suis* we use hamsters. When the hamster's temperature exceeds 40° C, the animal is sacrificed and kidney material used to inoculate the artificial medium.

DR CLARA RAVEN (United States) During my 2 years of service in the medical corps in France and Germany, 1945-46, 400 cases of infectious hepatitis were admitted to a U S Army general hospital.

separating the disease agent from other elements of the environment, and looking upon disease processes as the interaction of a triad—a host—and man is the primary concern—an agent of disease, and thirdly, the remaining inclusive features of environment.

Beyond the matter of survival, the health of an individual or of a species represents a dominance of positive adaptations to its particular environment, through greater numbers of positive than negative adaptations, or because they are more heavily weighted. Otherwise the organism can scarcely endure. Disease in terms of man is a negative resultant of the forces of ecology, the extent and seriousness of which is dependent upon the kind of balance—the nature of the biologic equilibrium—currently existing between human host and agent of disease. This is in every sense a varying equilibrium, and the costs of the negative adaptation are assessed in terms of the clinical nature of the disease that results, the number of persons affected, and the places and duration of the process. Epidemiology is medical ecology.

With the biologic adjustment between host and parasite fairly evenly weighted, with disturbances in equilibrium of limited degree a disease like bacillary dysentery results—from a world standpoint widespread and readily transmissible, epidemics relatively infrequent, not too exacting in terms of death and disability except for infants and the aged, and not too susceptible to measures of control. By contrast, cholera illustrates a less satisfactory adaptation between host and parasite. Rather favorable circumstances of environment are essential to its spread, the swings in disease prevalence are greater, and the cost in cases and deaths can be appreciable. This serves likewise to limit its current prevalence to relatively few parts of the world. Cholera has many times evidenced its ability to strike widely when favoring circumstances exist, but in the absence of their continuing presence it fails to establish itself. Cholera in Vermont in 1832 and the epidemic of Lake Champlain are little more than memories.

time, each in its proper perspective, and as they relate one to the other. Present day epidemiology tends to stress the importance of the agent of disease, largely by reason of the better methods and the greater ease with which this factor may be measured and evaluated.

#### AN INTERPRETATION OF ENVIRONMENT

The limited perspective with which environment is commonly viewed as an epidemiologic factor is improved by looking at environ-

# TROPICAL ENVIRONMENT AS AN INFLUENCE ON INFECTIOUS DISEASE

JOHN E. GORDON, M. D., *Professor of Preventive Medicine and Epidemiology, Harvard School of Public Health, Boston, Mass.*

An interpretation of environment, tropical or otherwise, as a simple matter of climate is incomplete and casual, especially if climate itself is resolved into a matter no more complex than relative admixtures of atmospheric temperature and humidity. Judgment as to the nature of a particular environment too often becomes wholly

cal factors by which one part of the world is so commonly distinguished from another. Many times this feature of environment has the greater force in determining the nature of man's existence, especially in relation to disease—what it is and where it tends to be.

Increasingly, communicable disease comes to be understood as conforming to the laws of ecology (2), with its distributions in time and space and its clinical nature the manifestations of a variable biologic equilibrium that involves two contending species, a host and an agent of disease. Thus the consideration of environment as a determining influence on diseases of man becomes more than the action of climate on the human host, which is the emphasis so commonly taken. The environmental influences exerted on the infectious agent can be equally significant. Similarly, the several elements of environment often act independently of the host and agent directly involved in the production of a disease, to determine in im

Ecology in its simplest terms deals with the relationship between

but with species and their interrelationships. Those relations are recognized (3) as particular, continuous, reciprocal, or indissoluble. Translated into terms of communicable disease, the kinds of infection are variously natural, foreign, refractory, accidental, or casual (4).

Health and disease, like the fundamental matters of existence and survival, are thus the resultants of an ecologic interplay (5). Because communicable disease is so evidently a matter of the reciprocal influence of two organisms, of a host and an infectious agent, the ecologic interpretation of disease phenomena is best accomplished by

the habits and customs of people, both those which are inherent or of natural evolution, and those arising artificially from religious or other tabu. Little quantitative information exists about the influence of education, clothing, income, and social welfare on disease of the tropics. The recently appreciated significance of psychologic effects and psychiatric well being has scarcely been extended to tropical medicine, otherwise than to the temporary and imported white populations. Much of the effect heretofore attributed to physical and biologic environment undoubtedly rests within this field of socio-economic environment, the most underdeveloped field of epidemiology, tropical or otherwise.

### A PATTERN FOR THE STUDY OF ENVIRONMENT

The ecologic approach to the study of communicable disease has

for the biologist in relation to the individual (9). It is an equally valid approach to the study of disease as it affects communities of people, that is to say, mass or herd disease.

Environment has been separated into three components. From this emerges a pattern for the study of the general environment as an influence on disease—a differentiation of six statistical cells into which may be set those phenomena attributable to the various elements of the three features of environment as each acts on host or on agent.

*Criteria for evaluation*—Two principal criteria exist for determining the effect exerted on disease by the several environmental components. The first is the observed variations in the clinical nature of the disease process. The second is the peculiarities of frequency distributions within time and space. One or other or both may be evidenced.

Diphtheria as a faucial infection is uncommon in the tropics, although its frequency as an infection of the skin suggests more at

basis under tropical and temperate conditions are noteworthy. Measles, typhoid fever, poliomyelitis, and others of the common infections of childhood show similar clinical modifications although the agent is as extensively prevalent in the one region as in the other.

Individual and peculiar distributions of communicable disease by reason of environmental effect are well known, numerous and precise. Oroya fever in certain valleys of Peru and at prescribed altitudes, a factor of the biologic environment, yellow fever, so strongly influenced by the physical factor of the environment, cholera, dominantly socio-

ment as composed of three major elements. the physical environment, the biologic environment, and the socio economic environment

Heat and humidity are accepted as dominant features of the physical environment of the tropics, by reason of such important actions as determine the local and general distributions of *glossina*, and hence of African trypanosomiasis. Other climatic influences receive less

weather. A difference in geologic structure was found by Buxton (6) to explain the freedom from filariasis of the east side of Espiritu Santo in contrast to heavy infection on the west. The east side was a porous coral chalk, the west side thick old volcanic soil with stand

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they have on disease prevalence in man. Repeated evidence is given of a direct action on one or the other member of the host parasite complex. Resistance of the human host is favorably or adversely altered, the action of the agent inhibited or enhanced. Under all circumstances the biologic environment is intimately related to the physical, for as the physical environment acts on man as a host, so likewise it affects these other hosts—to the extent that entomologists approach the problem of insect outbreaks (8) as epidemiologists study disease, ecologically.

The socio economic component of environment is that which relates to the association of man with his fellow man. In simplest terms, it is human ecology. The measurement and evaluation of the physical factors of environment is accomplished with some certainty. A progress in biologic matters has characterized the past century; but in social and economic influences on mass disease, not only is information grossly inadequate but satisfactory methods for study largely remain to be developed. A solid attack on the factor of nutrition is under-



clonorchiasis having been carried to all parts of the world by emigrants from the endemic areas of the Orient, no new focus has ever developed, because of the absence of appropriate snail hosts and the evident inability of the miracidium to use local snail species. This distribution has been further limited by factors of the social environment—the varying customs of eating fish raw or cooked—with the result that the disease is also variously absent in man in areas where infection in nature is great.

Transmission by arthropod vectors is so obviously a direct and important influence on the distributions of many diseases of man that

quently the distribution of the disease, is limited to warm countries. Conditions might well be otherwise were the bedbug a natural vector. Little need be said of numbers, a matter concerned in all biologic

perate regions and its essential absence in the tropics as an illustration of the influence of the socio economic environment on an agent of human disease. Explanation of the differences in behavior have been sought in the effect of light, temperature, and humidity. The action of the social environment is advanced as the more reasonable explanation. The customs and practices of tropical man lead to every

bohydrate favors multiplication of the agent and protein reduces it. The clinical change that takes place when the host transfers from tropical to temperate regions is as reasonably a function of this social environment-host factor of diet as of temperature and climate.

economic, and yaws similarly. Illustrations of the several categories of environmental influence on host and parasite now follow.

*Types of environmental action*—No single feature of the tropical environment has been accorded more attention than physical factors as they act on the human host. Curiously enough, this is largely related to the artificial host—the more or less temporary white resident—with little attention to the true native host. Despite the penetration into matters concerned with acclimatization of the unaccustomed, and the effects of the various physical components of the tropical environ-

of the pathogenesis of plague.

The frequency distributions of disease in the tropics are a common resultant of the effect of physical environment on disease agent. The yellow fever virus has well prescribed temperature limits within which it develops in the mosquito. It develops most rapidly and efficiently at 38° C, a 12 day interval is noted at temperatures of 25° to 28° C, and below 23° C infection does not follow (10). Well recognized isotherms have been established for the plasmodia of malaria. The distribution of filariasis is governed by the failure of the agent to pass the necessary developmental stage in the mosquito at temperatures ordinarily encountered north of 40° latitude. The vector exists at latitudes much beyond that limit. The agent is thus the susceptible part of the cycle through an influence of the physical rather than the biologic environment.

The number and complexity of the living things that surround man are alone sufficient to suggest the extensive influences of the biologic environment on health and disease of the human host. No particular search is necessary to demonstrate the action on specific infectious agents of human disease. Actual numbers are limited through invasion of nonsusceptible hosts, through ingestion as food or with feces, and through the action of agents are modified by

of a direct influence on the human host by biologic environment

and transmission of the agents of disease. Despite the parasite of

At all schools the incidence found among the boys was decidedly lower than among the girls of the same age group and prosperity. The negative correlation of age, the positive correlation with sex were clearly human material. There

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gether 4,114 healthy persons were examined bacteriologically, of whom 4.23 percent were found to carry diphtheria bacilli. Among the children of school age the incidence appeared to be 6 percent.

charge of inflamed conjunctivae in 4.3 percent, from the discharge of inflamed ears in 11.5 percent, and from ulcerating wounds in 31.1 percent.

It can be concluded that diphtheria as a manifest disease occurs only sporadically among native children in Batavia. The greater

in particular of the ears and wounds. Prosperity, age, and sex constitute the all-important factors on which the level of immunity depends.

Dr L. W. HACKETT (Argentina). Dr Gordon has stressed the communicable diseases in hot and humid climates. People who discuss tropical diseases often forget that the tropics are not all jungle. Much of the tropics is high in altitude, cool and dry. The most prevalent diseases are tuberculosis and the venereal diseases. Many conditions that have been eradicated from temperate zones, persist

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Dr KOUWENAAR (Sumatra): In Sumatra, the mortality from the tropical diseases is only about 5 percent. Many problems are unanswered. Why do we see so much cirrhosis of the liver and no scarlet fever? Such questions could be multiplied a hundredfold.

Dr GORDON (United States of America). I am indebted to Dr Hackett for reinforcing my thesis that latitude is not the sole determining factor in diseases of the tropics. I agree that noncommunicable diseases are now the most important. Nevertheless, ecology applies to more than communicable diseases.

## SUMMARY

Each of the factors of environment—physical biologic, and socio economic—has been considered individually as it acts on the host and on the agents of disease, to the end of demonstrating principle. But the illustrations themselves, and more particularly the definition of epidemiology as medical ecology, show this to be an oversimplification. All environmental factors are intimately interwoven, each influenced by the other. The production of disease in man is the resultant of the total forces within a universe—of an ecologic unity.

The principal advances in tropical medicine have been in clinical

in respect to herd reactions having as an objective that epidemiologic interpretation so largely accomplished for disease of temperate re-

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## ABSTRACT OF DISCUSSION

Dr J E DINGER (Netherlands) commentator. I should like to tell a few words at . . .  
 theria in a study w  
 out in 3,298 children  
 in children of school age is largely influenced by the standard of

island of Mactan (about 1 mile east of the city of Cebu), are very similar, and the statistics of the two communities therefore are combined in the presentation which follows

More than 99 percent of the enumerated population (15 530) of the two areas were given physical examinations. These examinations were detailed so far as the skin is concerned, the whole body being inspected except the pubic region in the female. Including patients in segregation, a total of 294 cases were discovered, or 18.3 cases per 1,000 of the population. More than one half of these cases were lepromatous. There was the usual excess among males, but this excess was observed only in the lepromatous type. Neural leprosy appeared to affect the sexes equally.

A feature of very great interest is that a substantial proportion (more than a quarter) of the cases were considered to be clinically inactive. The tendency to self healing in neural leprosy is, of course, well known, but it is not so well appreciated that occasionally in persons with no other signs of leprosy there are found inactive and apparently "healed" macules.

From the records it was possible to estimate the average annual attack (incidence) rates over the period of years covered by the household schedule (3). To do this conveniently a modified life table method was adopted which has been used in the study of other chronic

is, they are entered as of date of birth, if born in the household, or as of date of entry, if they entered the household through marriage or for other reasons. Similarly, they are removed as of date of death or departure. Only persons developing leprosy while living in a household of the surveyed communities are counted in the numerator.

On all the records, there were included 21,791 persons. Each of these, on the average, was in one of the two communities for 15.4

That is to say, 321.6 person years of exposure to leprosy, or 12 cases per 1,000 persons per year, on the average, for the period covered by the records.

of onset in leprosy is a serious error. Never rates for Cordova are as high as the high

even among persons over 50 years of age. Table 1 gives the specific rates for both communities combined.

## Session 6 LEPROSY

*Monday, May 17, 9 30 a m to 12 m*  
*Auditorium of National Museum*

### STUDIES ON THE EPIDEMIOLOGY OF LEPROSY

JAMES A. DOULL *Medical Director, United States Public  
Health Service*

In 1933 the Leonard Wood Memorial for the Eradication of Leprosy and the Bureau of Health of the Philippines agreed jointly to participate in field investigations of leprosy. These investigations were interrupted by the war but have recently been resumed. The findings here reported are based on the earlier data collected between 1933 and 1941.

The areas chosen were the municipalities of Cordova, Talisay, and Santander in the Province of Cebu (latitude 9°20" to 11°15" N longitude 123°20" to 124°5" E) in which as a whole leprosy had been unusually prevalent. A study by Dr. José Rodríguez (1) had provided leprosy prevalence rates for each municipality of the province, based upon the number of patients segregated over a period of 24 years. This study showed prevalence to be high in Cordova and Talisay and to be low in Santander. A preliminary survey by Rodríguez showed also that the inhabitants were unusually friendly and cooperative, considering that segregation of bacteriologically positive patients had been enforced for more than 25 years. Furthermore, church and municipal records of vital statistics were available which were of fundamental value.

The original objectives were simple (2). It was desired to learn first of all, and necessarily by physical examination of all the inhabitants, the true frequency of leprosy in these localities. At the same time it was hoped to obtain a fairly accurate history of each household. Such histories would permit the estimation of the attack rates which had prevailed in each community and in households in which exposure to various types of leprosy had occurred. Data were collected also regarding diet, prevalence of various types of insects, existence of other diseases, occupations, and other possibly pertinent matters.

sible after discovery, the lag between onset and discovery is well known to be a matter of months in most instances and sometimes of years. For both communities, there was included a total of 27,333 years of life experience of individuals subsequent to their first exposure to leprosy in the household. Among these persons there occurred 150 cases of leprosy, or an average attack rate of 5.3 cases per 1,000 person years.

For comparison, there was a total of 307,663 person years for individuals who had no record of exposure to leprosy in the household. Among these there occurred 252 cases, or an attack rate of only 0.8 per 1,000. Expressed as a ratio, the risk for the exposed group was more than 6 times that for those with no history of household exposure. This ratio was about the same for males as for females (table 2).

TABLE 2—Annual incidence of leprosy (all forms) based on family histories for those exposed in the household and for those not exposed by age and sex for Cordova and Tallian combined

Age—period of life experience (in years)	Attack rate per 1,000 person years					
	Exposed in household			Not exposed in household		
	Male	Female	Total	Male	Female	Total
0 to 4	0.68	0.00	0.36	0.03	0.00	0.01
5 to 9	10.89	0.03	8.59	.93	.62	2.18
10 to 14	14.60	9.00	14.76	2.34	1.91	2.36
15 to 19	11.68	7.91	9.77	2.15	1.07	1.60
20 to 24	6.60	3.93	5.23	1.34	.35	.64
25 to 29	1.21	2.01	1.65	1.07	.21	.64
30 to 34	2.04	1.15	1.56	.91	.46	.68
35 to 39	2.85	2.48	2.75	.82	.42	.62
Total (adjusted)	6.09	3.67	5.33	1.11	.55	.83

It was rather unexpected to find the peak of age incidence in the same age group for those exposed in the household as for those not subject to household exposure. In leprosy households the average age at onset, nevertheless, was much earlier, the age curve declines sharply after its peak. Among the nonexposed, on the other hand, the decline is gradual, cases continued to occur at more or less the same rate even in the later decades of life.

*Risk of household exposure in relation to type of primary case.*—In table 3 a comparison is made of attack rates in households in which the type of primary case was (a) cutaneous (lepromatous), (b) neural, or (c) unknown. The attack rate for nonexposed persons (d) is given for comparison.

The highest attack rate (6.23 per 1,000 person years) occurred in those exposed to lepromatous cases. When the primary cases were neural the rate was only 1.6. The risk for household associates exposed to lepromatous cases was about eight times that for persons

TABLE 1—Average annual incidence rates for Cordova and Talisy combined by sex and age

Age (in years)	Attack rates per 1 000 person years— Cordova and Talisy combined		
	Male	Female	Total
Under 5.	0.05	0.00	0.03
5 to 9	1.65	.94	1.25
10 to 14	3.74	2.43	3.12
15 to 19	3.12	1.6	2.37
20 to 29	2.01	.84	1.28
30 to 39	1.69	.38	.73
40 to 49	1.04	.59	.60
50 and over	.85	.89	.89
All ages (adjusted)	1.55	1.54	1.20

<sup>1</sup> For this and the following tables where adjusted rates are given they are based on the total life experience of both communities

*Relationship between prevalence as estimated from cumulated incidence rates and actual prevalence as determined in surveys*—If it be assumed that persons with leprosy do not die off at a significantly faster rate than the general population, and that incidence has remained more or less the same during the period, then prevalence at

1, after multiplication by the number of years in the respective class intervals, gives an expected prevalence rate of 39.3 per 1,000 at 25 years of age. This is remarkably close to the actual findings. At the time of the survey the prevalence rate for persons 20 to 29 years was found to be 39.6 per 1,000 for both communities. But if the cumulation be continued beyond 30 years of age the earlier disappearance of leprosy patients from the population, presumably by death, is evident from the fact that the expectancy is considerably higher than the actual prevalence which was found.

*Trend of the disease. Earlier versus later period*—An attempt was made to determine the trend of the disease by splitting the life experience into earlier and later periods (4). For the earlier, individuals born between 1896 and 1910 were selected and their life experience was included only to the year 1920. For the later period, those born between 1911 and 1925 were chosen and their experience was included to 1935. It was found that the first group had an average period of observation of 14.4 years and the second an average period of 13.8 years.

Considering only lepromatous leprosy, the attack rates for the earlier and later periods, respectively, were for males 1.6 and 0.9 per 1,000 person years and for females 0.8 and 0.3 per 1,000 person years. These figures indicate a downward trend of the disease.

*The risk of household exposure*—Although the segregation law requires the removal of bacteriologically positive cases as soon as pos-



between 10 and 15 years of age, 4.8; between 15 and 20 years, 3.0; and at ages over 20 years, only 1.3 per 1,000. The earlier the exposure, the greater the risk. This is a commonly accepted opinion

jects, and may be fondled by the leprous member of the family

A clear relationship was also demonstrated between age of exposure and age at which signs of leprosy were first noticed. Among children exposed at ages under 5 years, the majority at time of birth, no lep-  
romatous cases were detected before they reached 5 years of age. Between 5 and 10 years of age the annual incidence rate for these children averaged 7.9 per 1,000. The rate increased to a maximum of 17.8 per 1,000 at 10 to 15 years, and fell to 12.4 per 1,000 at 15 to 19 years. The rate for persons of 20 years and over who were exposed before 5 years of age was only 3.5 per 1,000. Thus the experience, at successive ages, of those exposed in infancy and early childhood in these households shows that the highest incidence of lepromatous

clear, therefore, that the determining factor is not merely presence in the household but the age at which exposure takes place

brother, or sister

#### SUMMARY

A review is presented of certain epidemiological features of leprosy as observed in the municipalities of Cordova and Talsay in the Province of Cebu, Philippine Islands. Segregation of bacteriologically positive patients had been compulsory in the Philippines for more than 25 years prior to commencement of these studies in 1933.

The risk of attack for persons exposed to leprosy in the household was found to be more than six times that for persons not known to

The risk was eight times when the primary case was when the primary case was

neural

The household was positive for those

not exposed, whereas the risk for those exposed to neural cases was only about twice that for persons who had not been subjected to exposure in their own households

TABLE 3—*Attack rates for leprosy per 1000 person-years according to type of primary and secondary case*

Type of leprosy in primary case	Type of leprosy in secondary case			
	Cutaneous	Neural	Unknown	Total
(a) Cutaneous	1	1		
(b) Neural			1	
(c) Unknown		1		
(d) All types				1
(e) None (remaining population)				1

NOTE.—Adjusted rates are given in parentheses

It is curious that when the primary case was lepromatous the risk of contracting lepromatous leprosy (438) was about  $2\frac{1}{2}$  times the risk of contracting the neural form (170), but when the primary case was neural the attack rates for the two types were about equal. Further data on this question are necessary, but it may be that there is a familial tendency toward the neural form.

males, the figure is actually 29 percent. For females it is 14 percent.

Restricting the discussion to the expected prevalence of lepromatous leprosy in persons exposed to lepromatous primary cases, cumulation of the rates to the age of 25 years yields a total of 23.5 percent. For females the cumulation is only 8 percent.

Probably it has never been appreciated that approximately one male in four would contract leprosy under these circumstances in Philippine communities. Of the approximate correctness of the figures there can be little doubt. It is unlikely that more than the actual number of cases would be recorded on the schedules. Un-

less, for all types of leprosy in males exposed in the household to lepromatous cases

*Influence of age at time of exposure*—A definite relationship was established between age at time of exposure and the probability of developing leprosy (5). When the experience was restricted to the occurrence of the lepromatous type among persons exposed to lepromatous leprosy in the household, it was observed that the average incidence rate for those exposed before 5 years of age was 7.6 per 1,000 per year, for those exposed between 5 and 10 years of age, 6.5,

The investigator might visit a village, ask the head man how many people lived there and how many were known to have leprosy, see a few patients and pass on to the next village. Or, a central office might attempt to ascertain the number of known cases by means of a questionnaire sent to all of the registered physicians of a country or district. Such methods may reveal something of the regional distribution of the disease, but little else.

In a few cases, the value has been found to be of much value. More information is needed, but in other cases, the value is not clear. The process of examination may be gained from local chiefs could be an examination, but in few places could anything like that be done.

The method employed by the Leonard Wood Memorial epidemiology unit in Cebu, set up and advised by Dr. Doull, was time consuming and expensive as it involved making a detailed census of the people in the region under examination, and examining them all. The method has never been employed elsewhere, and the statistical data gained are therefore unique. To what extent they may be applied to other regions and peoples cannot be said, for the variations are great. However, the conclusions regarding the age factor in leprosy, with general experience, not

The same is to be said of the method. It is to be hoped that such a study can be extended to permit answering the question of familial susceptibility, i.e., whether the actual members of a leprosy family are more susceptible than persons of other families living with them. Finally, the greater degree of danger from a case of the lepromatous type than from one of the neural type gives support to the practice of segregating only the former, though the fact that the statistical difference is not solved question ('closed').

*Classification*—The most controversial question today is how cases should be classified. It is a question of importance because—apart

household to lepromatous leprosy developed the disease before reaching the age of 25 years

These facts emphasize the peculiar danger which lurks in the im-

objects or even transmission by some insect of restricted mobility might give a similar picture of concentration around the infectious case

the hope that similar studies may be undertaken in other parts of the world in which leprosy is a problem

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### ABSTRACT OF DISCUSSION

Dr. H. W. W., ...

could not be present, the privilege has been given me of dealing briefly with that subject as well.

The investigation reported by Dr. Doull is unique. So called censuses and surveys of leprosy have varied widely in method and scope, but most of them have been of the superficial or "extensive" type.

the capacity to react in this way whereas lepromatous cases do not. Thus there is agreement with the histopathology, bacteriology, and prognosis of at least the cases of the "polar" types. The test is not diagnostic, but it is indicative of prognosis, hence the emphasis placed upon it by the proponents of the South American scheme of classification.

Dr C B LARA (Philippines). Apart from the failure of attempts at the experimental transmission of leprosy to adult man and the lower animals and in the cultivation of the *Mycobacterium leprae*, there has been difficulty in obtaining basic epidemiologic data. This is due to the prolonged and variable incubation period, great chronicity and variation in the manifestation of the disease, and its marked tendency to more or less complete spontaneous resolution, especially in its earliest stages.

An appreciation of some of the above mentioned difficulties led Dr C Manalang in Manila, since 17 years ago, to study the transmission of leprosy through a study of its pathogenesis from the early stages (in children of lepers) to eventual death or apparent arrest in the

ultramicroscopic or virus stage of *M. leprae*, the acid fast bacillus being the organisms. Therefore the organism is a transmitter as the (years) is susceptible, positive contact is held

to skin contact, to the sweat ducts of the latter's sweat ducts.

Much work needs to be done before Manalang's theory can be fully verified. More study of the morphology and biology of *M. leprae*

years, independent of those stimulated by the virus. They have yielded evidence tending to support some of his conclusions. Thus, among the children of lepers in Cullion, observed frequently from birth, a very large proportion at least 50 percent under age of 5 years, have undoubtedly lesions before the age of 5 years. In most

investigation of the house contacts of lepers

been accorded so important a role. In that scheme there were three

most workers in other parts of the world but at the Habana Congress in April 1948 an attempt was made to reconcile the opposing views. The proponents of the new scheme agreed to change the name "incharacteristic to indeterminate" and to reduction of that class from the status of "type" to "group" and the opponents—or a majority of them—made concessions in their turn. Only a part of the resultant scheme was accepted by the Congress in plenary session and the situation today is more confused than ever.

leprosy was one of the few diseases other than syphilis to give positive results that complement fixation tests with various bacterial antigens caused certain workers to call leprosy serum universally reacting. That many kinds of serological tests even the most refined ones for syphilis give positive results and that there is no diagnostic test

From the earliest days of the tuberculin reaction also special interest was taken

tuberculous leprosy patients than among comparable groups of normal people but the matter is of little practical importance. The many attempts to arrive at specific tests of this type with products of cultures derived from leprosy lesions (leprolins) have been quite unproductive.

References to immunology today pertain primarily to a skin test

(Dharmendra)—it gives rise in practically all definitely tuberculoid cases in many simple macular ones and in varying proportions of normal people to a papulonodular reaction lesion which begins to develop after some ten days on the average, sometimes goes on to

years or more. We have continued the observations of Doull and his coworkers. We believe as many as 40 percent of children who develop benign leprosy recover spontaneously and without treatment. Furthermore, age at exposure and contact with the lepromatous cases is of supreme importance in the serious forms of the disease. Of patients with lepromatous lesions and the prelepromatous macule (hazy patches of the Philippine workers) 75-85 percent give a history of contact within the same house with an open case. Further, we have evidence that the lepromin reaction bears a direct relationship to contact with an open case, as we have found the percentage of negative reactions in contacts increases in proportion to the closeness of contact with a lepromatous case.

It is on this basis that we have developed a preventive scheme, the principle of which is to segregate open cases from night contact with children. This can be done in rural areas in India, because, except for the weaving community, the villager is out in the fields most of the day. We therefore have set apart an area where all open cases from villages in the control area have to come and sleep at night. They are permitted to work in the fields in the daytime. There is some evidence over the past 6 years that the disease is decreasing in

3 years practice in the  
we have focused our  
interest on any possible influence of nutrition in the pathogenesis and  
epidemiology of leprosy. It appears that leprosy occurs more fre-  
quently among those people, and peoples, who eat plenty of semi-

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epidemiology of leprosy

remarks carry us back to the old fish theory of Hutchinson, which  
has been thoroughly exploded

he various leprosaria revealed that the probable source of the infection, usually the mother, had bacteriologically negative lesions in many cases

In the light of the foregoing observations, we feel that there is need for revaluation of the results of and the conclusions drawn from epidemiologic investigations that have been carried out along the usual pattern of such work

In about 200 cases among exposed children of lepers born in the Culion colony, the average age at onset of the disease was about 20 months. In most of these cases there has been a fairly rapid and of the lesions from the third to of the cases there has been no uned apparently free from the

disease for 5 to 14 years. Aside from minute scars in some cases the healing of the lesions is apparently complete, from histopathologic evidence. It remains to be determined, however, whether foci of the infection remain in the lymph nodes and other deep structures

The lepromin test has been carried out two to seven times in the past 10 years on our closely observed Culion children. While most of the children react in a positive reaction of 1 year a negative reaction

associated with concurrent or subsequent clinical deterioration or even a relapse

Of particular interest was the observation that repeated lepromin testing (with suitable, simultaneous controls) of children still free

have been found to react to the lepromin test in much the same manner as the exposed children of leprous parents. All the above cited evidence thus seems to indicate an upsurge of resistance above the age of 2 years, which continues to adolescence. Whether this development is a natural process or acquired, and whether it is general or specific as regards leprosy alone, remains to be investigated

Dr ROBERT G COCHRANE (India) I was very much interested in Dr Doull's excellent and clear presentation. Particularly as this supports our findings in India. We have a clinic for the study of child leprosy which has now been in existence for 12 years. We have 700 children on the roll, of whom about 120 are on treatment, the balance are on observations, and many have detailed records covering 10



the routine use of chaulmoogra oil in maximum tolerated doses as reported by Johansen (6), Faget (7) commented as follows "A smaller number of patients than usual were taking chaulmoogra oil treatments either by mouth or by intramuscular injection. Since

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 results from chaulmoogra oil and the sulfones over long periods of time were analyzed. Such a marked superior therapeutic action on the part of the sulfones was demonstrated, in early as well as far advanced cases of lepromatous leprosy, that chaulmoogra oil no longer seemed to have a place as a routine treatment for this type of the disease. Since lepromatous leprosy constitutes approximately 85 percent of the patient population at Carville and the remaining 15 percent either respond as well without treatment as with treatment or do as well with sulfones as with chaulmoogra oil, a change in routine treatment was indicated, and accomplished. Chaulmoogra oil, it appears, enjoys a lack of more effect of this opinion

and its derivatives are of little or no value" in leprosy.

It must not be inferred from this reversal of policy in treating leprosy that chaulmoogra oil is no longer considered to be of any value.

extent

chaulmoogra

alone. Also, leprologists who report the greatest success with chaulmoogra oil advocate intracutaneous administration of the drug. Since this method of administering the oil proved to be impractical in the Carville group of patients because of the tedious nature of the procedure, the associated pain, and the extensive skin involvement usually present, it might be that recent advances in injection technique, such as the hypodermic (9), might make intracutaneous treatment with chaulmoogra oil feasible.

### SULFONE DRUGS

The use of promin, diasone, and promizole in the treatment of leprosy has been quite extensively reported in the world medical literature. When first reported (10) promin was regarded to be therapeutically more effective in leprosy than any treatment previously tried at Carville. This opinion still prevails not only for promin but for diasone and promizole as well.

## STUDIES ON THE THERAPY OF LEPROSY

FREDERICK A. JOHANSEN, M D, F A C P,<sup>1</sup> PAUL T. ERICKSON, M D,<sup>2</sup>  
*U S Marine Hospital (National Leprosarium), Carville, La*

Of a score or more experimental treatments employed in human leprosy at Carville only two, chaulmoogra oil and the sulfone drugs, have proved worthy of more than passing consideration. Chaulmoogra oil, of course, had been an established treatment in leprosy long before its use in this country. It was considered experimental only in the sense that it had not had an extensive and critical clinical trial in this country prior to its use at Carville. The sulfones, promin, diasone, and promizole, on the other hand, were first used in the treatment of leprosy at the National Leprosarium. They are now gradually becoming recognized in other countries as efficient therapeutic

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### CHAULMOOGRA OIL

The experience with chaulmoogra oil might best be summarized

Chaulmoogra oil is being continued in a large group of patients, and while no spectacular results have been obtained either with the oral administration of the crude oil or the intramuscular injections of its ethyl esters, it appears that definite improvement has followed in a sufficiently large percentage of cases to encourage the patients in the continuation of the treatment. Hasseltine (4) in 1938 stated "A

ment of leprosy, said 'Although there was no further evidence of definite specific action, the impression persists that the chaulmoogra oil products are of some benefit in leprosy.' In 1946, after the experimental use of the sulfones for a period of 5 years side by side with

<sup>1</sup> Medical director U S Public Health Service medical officer in charge

<sup>2</sup> Senior surgeon U S Public Health Service

injected into rats can still be found 18 months later retaining their acid fastness. It is possible that dead bacilli may remain identified in the skin for several years following recession of specific leprous lesions from treatment.

Rest periods from drug administration are observed every third week in the case of promin and for a period of 2 weeks every 2 months in the case of diasone. Although it might be expected that drug sensitivity would be produced by such a routine, this has not been our experience. Blood and urine sulfone level determinations have proved that rest periods are not only desirable but necessary to avoid toxic manifestations.

Unusually high concentrations of promin in the blood and urine have been found in several patients 9 days following the last dose of the drug, and one patient who had taken the drug for 6 years had a urine concentration of 0.7 milligram percent after an enforced rest period of 4 weeks during which time no sulfone or sulfa drug had been administered (14). It has been shown in the case of sulphetrone, a related sulfone, that greater concentrations of the drug were encountered in the skin in some cases than in the blood stream (15). It can be assumed from this observation that other sulfones as well, such as promin, diasone and promizole, are stored in the skin, and it is possible also that the liver acts as a storage reservoir. Rest periods presumably allow the release of sulfones from storage depots before critical levels are reached.

Another essential feature in the proper administration of the sulfones is to initiate treatment with comparatively small doses. The initial dose of promin should usually not exceed 1 gram and that for diasone 0.3 gram. A period of 2 to 4 weeks should elapse before the maximum dose is attained. Initiating treatment with maximum doses

occurred in some of our patients. These occurrences were undoubtedly sensitivity reactions to promin and not lepra fever. All of these patients received initial doses (0.01 to 0.1 gram) have developed hematuria without crystalluria. This was not occurred after initial doses were kept low. Also, a large number of patients are apt to develop gastric intolerance if initiated on maximum doses of diasone before tolerance to the drug has been developed.

It has been argued that *M. lepra* is apt to develop resistance against the sulfones if treatment is not initiated with maximum doses and such

Promin, diasone, and promizole are all derivatives of diamino diphenyl sulfone. It appears, but it is not definitely established, that diamino diphenyl sulphone, the chemical group common to all these drugs, is the active principle.

Promizole is synthesized with much difficulty (11), and because of this fact and because it shows no therapeutic superiority over the other sulfones in leprosy (12), its use may not be extended beyond present commitments, and further discussion of this drug will not be attempted here.

Rapid or spectacular cures are not seen from the use of sulfone drugs nor are they claimed to be specific remedies. On the contrary, they work slowly. Definite objective clinical improvement does not appear until after 3 to 6 months of treatment. As a rule, this is first noticeable in mucous membrane lesions, then in skin lesions, followed by an exceedingly slow reduction of *M. leprae* in these lesions as demonstrated in skin and mucous membrane smears. Improvement in these features of the disease are progressive, with few if any relapses. Evidence has also accumulated over a period of years that bone lesions presumably due to the direct action of *M. leprae* heal more slowly. Improvement of neural lesions (13). The most remarkable or unusual feature, however, to those who are acquainted with the unfavorable progression of the disease in many cases under chaulmoogra oil treatment, is the almost universal improvement seen under the sulfones and the fact that the disease seldom, if ever, appears to become worse.

From toxicity studies and blood and urine sulfone level determinations, when correlated with therapeutic effects obtained, it has been found that a daily dose of 50 grams intravenously in the case of promin and 10 gram orally in the case of diasone consistently gives good results. In general, the rapidity of objective clinical improvement is in direct proportion to the intensity of treatment, large doses producing faster regression of nodules, infiltrations and ulcerations than low doses. Individual variation to this rule, however, has been

leprosy lesions does not seem to be appreciably accelerated by large doses of the drugs. This has been demonstrated in a group of 10 patients treated intensively with doses ranging from 7.5 to 15 grams

by as much as 2 months. This may be clearer to our understanding when it is recalled that human leprosy bacilli killed by boiling and

suppressive effect on experimental tuberculosis in the guinea pig, a study of what value streptomycin might have in clinical leprosy was undertaken by Faget et al (19)

Ten cases of lepromatous leprosy were subjected to intramuscular doses for 11 months, two cases for 8 months and three cases for 7 months, two cases had treatment intermittently at first, because of sensitivity to the drug, after which one was able to continue with full doses for 11 months the other tolerated only 0.5 to 1 gram daily for 6 months. In addition to streptomycin, five of these patients received sulfone treatment, four promin and one diasone. The four who received promin had previously been on that drug for several months

of the patients under treatment, two remained stationary, and one became slightly worse. Nasal obstruction and epistaxis were checked in a few cases and healing of a leprosy ulcer of the soft palate occurred rather rapidly in one patient. The improvement that did occur all happened during the first 2 or 3 months of treatment. After this the condition of the patients remained stationary and that of one became worse. It cannot be definitely said that the improvement noted was more rapid in those patients who were also taking sulfones, than in those who were not.

Partial relief was sustained after 3 weeks on a dose of 1 gram streptomycin for scabbing lesions of the mucous membrane of the nose which had been causing obstruction to breathing and epistaxis over a period of years. The acute symptoms of pain and iridocyclitis has also been relieved in patients to whom streptomycin was given.

Among the side effects, rashes were severe and frequent in all patients, malaise and fever, skin eruptions were troublesome and impaired hearing occurred in its still complain of vertigo, especially in going from a light to a dark place, 11, 14, 14, and 15 months respectively after discontinuation of streptomycin. Eosinophilia was unusually intense, varying from a low of 5 percent to individual heights of 42, 43, 47, 54, and 65 percent, commencing within

on maximum doses of promin as routinely given with the doses later

continuous in spite of rest periods and thus deter development of resistance of the organism against these drugs

Red and white blood counts and urinalyses have been performed on our patients at regular 3 week intervals This has been essential

followed These laboratory tests can possibly be eliminated, except perhaps during the first month of treatment, provided iron therapy is given routinely

Rest periods and proper mutual dosages, it is felt, have materially contributed to the low toxicity record experienced at Carville from the use of sulfone drugs When it is considered that a number of our patients have taken as much as 10 pounds of promin over a period of 6 years without a single toxic reaction except a low grade anemia, the apparent innocuousness of these drugs when properly administered can be readily appreciated Unlikely as they are to produce toxic effects, they should not be abused by pushing dosage to the limit Good therapeutic results have been observed on comparatively small doses This suggests that minimal effective dose determinations should be ascertained where cost of drugs is of paramount importance

The mode of action of the sulfone drugs is not definitely known It has been felt that the diamino diphenyl sulfone radical is the active principle which produces a bacteriostatic action upon *M lepra*. An other belief is that these drugs depress the red blood cell count

#### STREPTOMYCIN

After 1 ann - 3 3 3 3

therapeutic measures were invariably disastrous. Improvements cured would subsequently succumb to further progression or relapse of the disease. Interest has been revived in such procedures for relief and prevention of physical deformities by early treatment supported by whatever corrective measures are at doctor's disposal.

It is felt without equivocation that the sulfones must be regarded the treatment of choice at present for leprosy in this country. The drugs, however, are not the complete answer to the treatment problem in leprosy. Further search should be made for quicker acting therapeutic agents. Antibiotics having a demonstrable bacteriostatic effect on acid fast organisms warrant further investigation, as do new and related drugs of the sulfone series.

### CONCLUSIONS

Sulfone drugs have been found to be an effective treatment for leprosy. Their therapeutic action is considered to be superior to chaulmoogra oil and its derivatives administered in maximum tolerated doses intramuscularly and orally.

To secure the best therapeutic results with a minimum of toxic effect, sulfone treatment should be initiated with small doses which are gradually increased as tolerance is developed, and rest periods should be observed.

Increased cost of sulfone drugs over chaulmoogra oil is largely mitigated by their reducing or making unnecessary expenditure for the care of complications associated with the disease. Determination of minimal effective doses is of value where cost of drugs is of paramount importance.

Streptomycin and other antibiotics having a demonstrable bacteriostatic effect on acid fast organisms warrant further trial in leprosy, as do new and related drugs of the sulfone series.

Although not considered specific remedies, sulfone drugs must be regarded the treatment of choice for leprosy at present.

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Streptomycin, in large and continuous doses produces toxic manifestations too severe in comparison with results obtained. Unless this disadvantage can be overcome, streptomycin must be classed as of doubtful value for systemic use in leprosy. In low concentrations it has been found to be of value when used locally as a solution or in a water soluble ointment base on leprosy and trophic ulcerations (20). Local irritation may occur in retreated cases unless low concentrations are employed.

#### DISCUSSION

The improved status in which the patients at Caryville find them- of the chaulmoogra oil days  
superiority of  
leprosy. Im-  
been of Eu-  
ropean, American, or Oriental extraction. Sufficient saving has been accomplished in the decreased need for bandages and materials for the proper care of ulcers to cover the cost of the new drugs employed. Only one tracheotomy has been performed during the sulfone regime, and this was on a patient as yet not treated with a sulfone. Tracheotomy during the chaulmoogra oil days was a rather frequent procedure.

Since the chaulmoogra oil days, the most common method of treatment that

where they were formerly absent, and erasure of cicatricial and redundant distortions of the face are the most common reconstructions attempted.

During chaulmoogra oil days results from orthopedic and physio



## NEW DEVELOPMENTS IN THE THERAPY OF LEPROSY

R G COCHRAN, *Medical Secretary, Mission to Lepers, Honorary Director Leprosy Campaign, Madras, Honorary Medical Superintendent, Lady Willingdon Leprosy Sanatorium, Chingleput, Lecturer in Leprosy and Dermatology, Christian Medical College Vellore, South India*

I appreciate the honor and privilege of presenting this paper on "New Developments in the Therapy of Leprosy" and would remind

administer, is of little practical value except as a guide to further research

represents that form of progressive disease in which the tissues of the body are unable to organize an effective defense. I believe that without active multiplication of the *My leprae* in the corium of the skin it is impossible to develop lepromatous leprosy. This, therefore, means that the strategic point of attack against the *My leprae* is in the cutaneous tissues. Hence a drug to be effective must either be injected into the corium, or be concentrated in sufficient quantities to

therapy was dependent on the dosage of the drug and on its proper administration

Further, this authority states that he believes that any method of hydriocarpus therapy which does not include intradermal injections, or in which the patient receives less than 400 cubic centimeters of the

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diaminodiphenyl sulphone derivatives are effective in lepromatous leprosy. While these remedies show marked clinical improvement in this type, the bacteriologic improvement is not commensurate, and is very much slower.

**Diasone (diaminodiphenyl sulphone formaldehyde sulphonate)** — The majority of cases showed significant clinical improvement, but in 8 out of 30 cases the clinical condition was either stationary or worse. The bacteriologic condition showed no improvement or had deteriorated in 12 out of 30. Admittedly the average period, 19 months, was too short for definite conclusions to be drawn. No case had become bacteriologically negative in this period. We noted, however, that in the dosages given there was a marked tendency, 18 out of 80, for the drug to precipitate lepra reaction with erythema nodosum like lesions. In the majority of cases these reactions subsided when treatment was continued, in 4 the condition was severe, and 3 had to discontinue diasone because of the persistence of reaction.

**Sulphetrone (diaminodiphenyl sulphone phenylpropylammonotetra sodium sulphonate)** —We agree with the opinion expressed by Wharton (1947) that sulphetrone appears to be more rapid in action and less liable to produce lepra reaction than any other member of the sulphone group of drugs at present in general use. Out of nine cases, in only one was the reaction condition severe enough to necessitate the discontinuance of treatment. Eight cases were much improved in the clinical condition and in their bacteriologic state, while in one the bacteriologic state deteriorated and the clinical condition remained stationary. The average period in which these results were obtained was 1 month, compared to 17 with diasones. The dosages of sulphetrone compared with those of diasones are given in the following table. The dosages of sulphetrone compared with those of diasones are given in the following table.

Therefore, apart from the inconvenience of taking large doses, sulphetrone appears to be, at present, the sulphone derivative of choice

In the sulfone drugs we have a new and powerful remedy for advanced and moderately advanced lepromatous leprosy, but in India

Therefore, it is recommended that for the present sulphone therapy we

national Congress of Leprosy, I presented evidence that, as far as our cases in India were concerned, by intensive intradermal injections combined with subcutaneous injections of hydnocarpus oil in a dosage of 15 cubic centimeters per week 50-3 percent of our early lepromatous standards are reached. It is international.

In reviewing modern therapy in leprosy, it must be admitted that even the most enthusiastic advocates of hydnocarpus oil have not been altogether satisfied and have viewed with considerable uneasiness the high rate of relapse after recovery. To these workers the relative lack of success of the hydnocarpus remedies in these cases, and in

racial groups than in the Indian or African. The modern advances in sulphone therapy are, therefore, greatly welcomed. In discussing these remedies, however, a sense of perspective must be maintained lest there should be a repetition of the uncritical enthusiasm of 25

phone. This substance has been known for many years, but up to now has been considered too toxic for human use. Feldman and Hinshaw (1940) reported the effectiveness of promin, a derivative of diaminodiphenyl sulphone, in the treatment of experimental tuberculosis. This was followed by reports by Faget et al (1943) that promin had a definite action on the the *M. leprae* and successful results were claimed in lepromatous leprosy. This earlier work was followed by further publications by Faget et al (1945 and 1947), Fernandez (1946), and Muir (1947) both on promin and on another derivative of diaminodiphenyl sulphone, diasone. Wharton (1947) reported on a new derivative, sulphetrone, and claimed that it was less toxic and more effective than either promin or diasone.

In order to endeavour to evaluate the present position of the sulphone remedies I shall briefly discuss the experimental work we have done in Madras, and then pass on to what may be important further developments in the administration of these drugs. We have been investigating the place of sulphones in the therapy of leprosy for

advanced lepromatous case, with the definite possibility of complete relief of the distressing complications associated with lesions of the nose and throat, the present methods of administration have certain definite disadvantages. These are (1) Oral administration is unsatisfactory, because the exact amount of the drug absorbed cannot

be ascertained, (2) It needs trained personnel not readily available in India and the East, and it usually results in rapid absorption, but with equally rapid excretion.

It is surely logical to expect a drug for leprosy treatment to be sold at a reasonable price, and possible of administration in a practical but economical way. Oral administration is extravagant—intravenous medication, costly.

For the above reasons, and because we believe that the corium of the skin is a strategic point of the attack against *M. leprae*, we have searched for alternative methods of administration. The first modification of sulphone therapy was by intradermal injection. We used

'phone (15 percent) as a

In order to test the efficacy

the concentration of sul

phones in the skin, expressing our results in milligrammes per gramme of skin tissue. For comparison we estimated the sulphone content of

Experiment started by giving a 25 percent suspension of 4-aminodiphenyl sulphone in arachis oil (ground nut oil) by subcutaneous injection. Later when a suspension of pure sulphone in (ground nut) oil

reserved for the more advanced lepromatous cases, for those cases

of advanced lepromatous cases after a year's treatment, this applies particularly to nasal and laryngeal lesions, bacteriologic improvement is much slower and negative results cannot be expected under 3 to 4 years. There is still some doubt whether a significant number of advanced lepromatous cases become negative even after this period. Further, all patients on sulphone therapy should be warned that a

maximum of 6 tablets per day is reached. In the case of sulphatrazine, our

at their maximum dosages for prolonged periods, one year or more, without intervals for rest unless there are signs of intolerance. These

Similarly, the hope held out that streptomycin might be more effective in leprosy has not been sustained. Not only does it appear, so I have been told, not to be as effective as the sulphones, but its toxicity in the

It is evident that the advances in chemotherapeutic and antibiotic substances hold out great promise that at long last the therapeutic conquest of lepromatous leprosy may be within sight. In making such a statement, however, the temptation to excessive optimism must be resisted, and, therefore, the leprologist is urged not to discard the hydnocarpus remedies but to continue to search for more effective chemotherapeutic and antibiotic agents, ever bearing in mind that no remedy will be of ultimate avail unless practical of administration and reasonable in price.

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due to tissue damage has not been sufficiently stressed. To cure leprosy and permit patients to be ostracised, a drag on society, a misery to themselves, physical and mental wrecks, is no credit to us or to the society in which we live.

#### ACKNOWLEDGMENTS

Wellington  
 Hospital, and  
 quarters  
 To Dr

K. Ramanujan, the Assistant Director, Leprosy Campaign, my special thanks are due for his assistance at all times and for his many valuable suggestions. Dr C. G. Pandit, Director, King Institute, Guindy, has always been very ready to assist us with advice for which I am particularly grateful.

The Biological Department of the Imperial Chemical Industries, Ltd., has kindly supplied the suspension of  
 Similarly

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We are well aware of the dangers of using diaminodiphenyl sulphone suspensions, and, therefore, until more work is done we are not ready to recommend extensive trials of this drug. The above dosages, however, appear to be well within the limits of toxicity, for in no case did the blood concentrations rise above 2 milligrams percent. Our preliminary results furnish evidence that not only are these remedies effective, but a very much smaller dose is required for equivalent and in some cases more marked clinical and bacteriologic results, (70 grams of diaminodiphenyl sulphone as compared to 2,470 grams of sulphetrone, and 700 grams of diasone) and the average time taken to effect these results is shorter. The average time taken with the sulphone suspensions was 11 months as compared to 16 months with sulphetrone. We have reason to believe that emulsions of sulphetrone in arachis oil will give equivalent results with much less danger of toxic complications. It seems, therefore, appropriate to recommend that this line of development in sulphone therapy be further investigated. I am of opinion that if our findings are confirmed some of the disadvantages of sulphone therapy will be eliminated.

Another serious drawback to sulphone therapy, as has already been mentioned, is the tendency to the precipitation of lepra reactions in the early months. Sometimes this distressing complication is so severe that this therapy has to be abandoned.

Recent work by Wharton and the Carville workers indicates that certain antihistaminic drugs when given along with sulphones, may control this condition. Such a discovery would be of the greatest importance for it would bring sulphone therapy within the reach of the most active lepromatous cases.

No account of the development of therapy in leprosy would be complete without reference to the increasing importance given to the possible additive (synergistic) effects of a combination of remedies. While streptomycin has been discarded for prolonged use in leprosy, it may be found useful in this connection if given during rest periods in sulphone therapy, for not more than 1 month, and in smaller dosages. In this connection, the recent work of Feldman

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precipitate a lepra reaction of the erythema nodosum type, and, therefore, may prove of value if such reactions can be controlled



tention is being turned to the possibilities of combined use of different drugs, certain workers are using, among other things, combinations of sulfones, and a few of them combinations of chaulmoogra and a sulfone

From the practical aspect the present situation arouses some apprehension on the part of those who cannot employ the new drugs for routine mass treatment, but have to rely upon the old chaulmoogra (hydnocarpus) preparations—and see real value in them, when properly employed. It would be most unfortunate if the propaganda for the new drugs in medical and lay publications should serve to dis-

... chaulmoogra must still be relied upon  
the last 2 years we have treated 52  
ults have been excellent. I should

like to stress also the social aspects of leprosy. With the more effective therapy now available, I recommend a propaganda campaign so that patients will seek medical advice. The cost of the sulfones is still high and I should like to ask this meeting to suggest to the International Leprosy Association that an international formula be reached so there will be a uniform price throughout the world.

Dr R. G. COCHRANE (India). Both Dr Johansen and the other speakers who have given almost unqualified support to sulfone therapy have been working under conditions which have not been favorable to the administration of chaulmoogra oil. The majority of cases which these workers have treated are advanced lepromatous. Dr Sloan of Hawaii, from his reference to the necessity of tracheotomy, has evidently been dealing with advanced lepromatous leprosy. I believe that no one disputes the supremacy of the sulfones in advanced lepromatous leprosy. To judge the effectiveness of a remedy by the enthusiasm of the patients is deceptive because whenever good results are reported patients who have suffered years from a chronic disease are liable to lose their sense of balance. Nevertheless, while one would use the sulfones wherever possible, care must be taken not to

... for widespread use of these drugs in India  
which will be almost impossible  
... Conference that propaganda  
is a two edged weapon. Until  
methods of administration are discovered which are more suitable for  
mass treatment and the cost of the drugs is within reach of all

is unsound, treatment may be a valuable adjunct to prevention; the keynote must always be the prevention of contact of open cases with healthy persons particularly children

ment with the sulfone group of drugs

Cochrane R. G. J. Christian Med Assoc of India Burma & Ceylon 22 211 1947  
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# ABSTRACT OF DISCUSSIONS

Dr N R SLOAN (Hawaii) At Kalapapa on the island of Molokai we have used sulfones for about 2 years. I agree with all that has been said about their value. One good evidence is the attitude of the patients themselves. They have ulcers of years standing cleared up within several months, tracheal tubes removed, visual failure arrested—and they are enthusiastic. We used to do 8–15 tracheotomies a year, but did our last one a year ago. In the last year four children, 6–14 years of age, have been brought in by relatives on the

Dr H W WADE (United States) The present situation in the therapy of leprosy is unparalleled in that no one who has employed the sulfones has rendered an unfavorable report. At every center visited in a recent tour of South America there was nothing less than enthusiasm, on the part of the patients as well as the physicians. Much emphasis was laid on the rapid amelioration of lesions of the mucous membrane of the nose and throat, also the

which began about 7 years ago. Dr Lauro de Souza Lima, of the Padre Bento leprosarium in São Paulo, is second as regards time and first as regards numbers of patients treated—a total of 1,287 since 4½ years ago. According to his report at the Habana Congress, in no case under treatment had the disease progressed, less than 4 percent of 841 lepromatous cases had failed to improve in some degree, and in 23 percent the lesions had cleared up (in 66 percent of 99 “incipient” cases). He pointed out, however, that not a few cases improve only to a certain point and then become stationary. Also that bacteriological improvement does not parallel the clinical improvement. In 50 percent of 150 biopsy specimens from the sites of subsided lesions bacilli—more or less greatly modified in appearance—were still to be found. The whole experience

reached. More rapidly acting drugs are needed, or drugs with higher ultimate effectiveness, or more effective methods of treatment. At

## SECTION IV

# Virus and Rickettsial Diseases

### *Session 1. VIRUSES IN GENERAL*

*Monday, May 10—2:15 to 4:10 p. m*

*Auditorium of National Museum*

The meeting was called to order by Dr. John R. Paul, convener, with a word of welcome to those present and a brief review of the pertinent parts of the rules of procedure which were adopted at the opening plenary session. Dr. Paul introduced Dr. James W. Colbert, Jr., assistant secretary, and requested the speakers to turn over their manuscripts to him. Officers were then elected to the three positions of chairman and vice chairmen. The officers of the section were:

Chairman: Dr. John R. Paul  
Vice Chairman: Dr. James W. Colbert, Jr.  
Secretary: Dr. James W. Colbert, Jr.

The following papers were presented to the section:

I accept Dr Wade's remark about the change of tissue reaction from leproma to tuberculoid with a certain amount of mental reservation. Personally, I should not be willing, in as important a matter as this, to accept the evidence on a clinical history that a case had been previously leproma, without seeing the section from the case

but healthy skeptical attitude

DR J S K BOYD (United Kingdom) All of the work done on the sulfones has been done on human beings. No one has yet cultivated the leprosy bacillus, nor have we been able to infect experimental animals. This is a highly important subject, and one that should receive extensive study.

#### CLOSING REMARKS OF THE CHAIRMAN

DR J S K BOYD It is now my sad lot to make the closing remarks of this section. I am sure that I express the general attitude as I thank all of the speakers. We have enjoyed their papers and have benefited from them. We also have profited from the more intimate and more informal discussion, as well as from the personal contacts we have made or solidified.

I wish to express my thanks to the vice chairmen of this section, Dr Meyer and Dr Sokhey. I also wish to thank Dr Turner, our con-  
vener and able secretary, and his assistants, Dr Reynolds and Mrs Smith. And now, it's time to say—Farewell and till we meet again.

spotted wilt, tomato bushy stunt, corn mosaic, cucumber mosaic, and sugar cane yellow stripe. Bacteriophages, which are agents capable of causing the lysis of bacteria, are now regarded as viruses.

The viruses have been separated as a special group of infectious, disease producing agents by means of several general properties, no one of which is, however, exclusively characteristic of viruses. Nevertheless, no great amount of difficulty has been encountered in the segregation of the virus group. Viruses are characterized by their small size, by their ability to reproduce or multiply when within the living cells of a given host, by their ability to change or mutate during multiplication, and by their inability to reproduce or grow on artificial media or in the absence of specific living cells. The sole means of recognizing the existence of a virus is provided by the multiplication of the virus, which is, of course, usually accompanied by manifestations of disease. Viruses spread from diseased to normal susceptible hosts by different methods. Some are transferred by direct contact, as when a diseased leaf is caused to rub against a healthy leaf by a gust of wind, or when a normal person or animal comes into direct contact with a diseased person or animal. Such viruses can usually be spread by

In some cases a highly specific intermediate host is necessary, and a more or less definite period of incubation within this host may be required before it can pass on the virus.

Reproduction, mutation, and metabolic activity have long been regarded as unique and special properties of living organisms. When viruses were found to possess the ability to reproduce and to mutate, there was a definite tendency to regard them as very small living organisms, despite the fact that the question of metabolic activity remained unanswered. Because of their small size they could not be seen by means of the ordinary light microscope. Although this fact puzzled some investigators, it was pushed aside, and for over 30 years' interest in virus research was centered about the discovery of new viruses and on studies of the pathological manifestations of viruses. Around 1930 Elford began his important work on the filtration of viruses through graded collodion membranes. He demonstrated that different viruses possessed different and characteristic sizes, and that some viruses were as large as about 300  $m\mu$ , whereas others were as small as 10  $m\mu$ . It was soon realized that the acceptance

and digestion and the general metabolic functions usually associated with life could be contained within structures as small as 10  $m\mu$ , especially since protein molecules larger than 10  $m\mu$  were known. It can be seen from figure 1, which illustrates the relative sizes of several

## THE NATURE OF VIRUSES

WENDELL M. STANLEY, *The Rockefeller Institute for Medical Research,  
Princeton, N. J.*

cepted. The cause of infectious disease remained a mystery for hundreds of years. Even the wonderful work of Leeuwenhoek and his description of small animals and bacteria during the years from 1676 to 1683 failed to result in proof of the relationship between bacteria and infectious disease. There was, of course, much speculation, and during the latter half of the nineteenth century great controversies arose over the germ theory of disease. Then through the brilliant work of Pasteur, Koch, Cohn, Davaine, and others, it was proved

Thus, when in 1892 Iwanowski discovered that the juice of a plant diseased with tobacco mosaic remained infectious after being passed

observations failed to attract attention. However, 6 years later, the filtration experiment was repeated and extended, independently, by Beijerinck, who immediately recognized the significance of the results

Plant  
virus diseases include tobacco mosaic, peach yellows, aster yellows,  
potato yellow dwarf, alfalfa mosaic, curly top of sugar beets, tomato

viruses and certain reference materials, that the viruses overlap with respect to size, not only with protein molecules but also at the other extreme with accepted living organisms. For example, several viruses are smaller than certain hemocyanin protein molecules, and several viruses are larger than the pleuropneumonia organism, which is an accepted living organism capable of growth on artificial media. The fact that, with respect to size, the viruses overlapped with the organisms of the biologist at one extreme and with the molecules of the chemist at the other only served to heighten the mystery regarding the nature of viruses. It became obvious that a sharp line dividing living from nonliving things could not be drawn, and this fact served to add fuel for discussion of the age old question 'What is life?'

Attempts to learn something about the nature of viruses through studies on their general properties began with Beijerinck's work in 1898 and were continued in different laboratories for over 30 years without too much success.

Important contributions, particularly of Vinson and Petre during

the 1920's, showed that tobacco mosaic virus could be subjected to several kinds of chemical manipulations without loss of virus activity. Nevertheless, in 1932 the true nature of viruses was a complete mystery. It was not known whether they were inorganic, carbohydrate, hydrocarbon, lipid, protein, or organismal in nature. It became necessary, therefore, to conduct experiments which would yield information of a definite nature. Tobacco mosaic virus was selected for these initial experiments because it appeared to provide several unusual advantages. Large amounts of highly infectious starting material were readily available, and the virus was known to be unusually stable. Furthermore, it was possible to titrate or measure the amount of this virus in a preparation with ease and rapidity and with great accuracy. During the course of a wide variety of early exploratory experiments, it was found that the enzyme pepsin inactivated tobacco mosaic virus only under conditions under which pepsin is active as a proteolytic agent. It was concluded that tobacco mosaic virus is a protein or very closely associated with a protein which could be hydrolyzed by pepsin. With this as a lead, efforts were made to concentrate and purify tobacco mosaic virus by means of the methods previously employed in work with proteins. By means of a combination of procedures involving salting out, isoelectric precipitation, and adsorption on and elution from an inert material, a crystalline material was obtained which possessed the properties of tobacco mosaic virus. This crystalline material was found to be a nucleoprotein with rod shaped molecules or particles about 280 m $\mu$  by 15 m $\mu$  in size and with a molecular weight of about 40,000,000. Early skepticism that a virus could exist in the form of a crystallizable nucleoprotein has largely disappeared, chiefly because of the vast

## APPROXIMATE SIZES OF VIRUSES AND REFERENCE MATERIALS

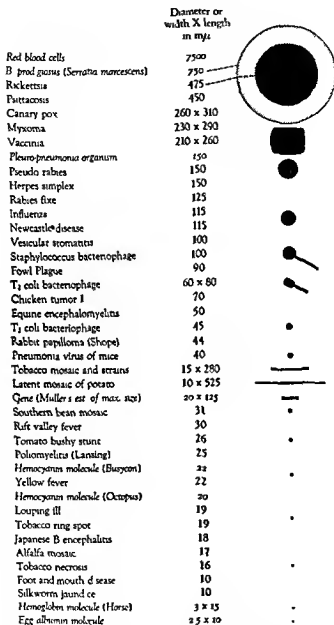


Figure 1.—Approximate sizes of several viruses and reference materials. From W. M. Stanley, *Chem. and Engin. News* 25: 3786, 1917.



large particles consisting of nucleoprotein, lipid, and carbohydrate and possessing in some cases a degree of morphological differentiation characteristic of organisms. Still other viruses have as yet defied isolation and purification, possibly in some cases because of extreme instability. Electron micrographs of several viruses have been obtained, and eight of these are shown in figure 2. The viruses that have been purified form an almost continuous spectrum of sizes and shapes. The smaller rod or spherically shaped viruses appear to be simple nucleoproteins, some of which can be obtained in crystalline form. The e appear to have chemical and physical properties which, neglecting virus activity, would tend to place them in the molecular world. The larger viruses have a composition and properties which are characteristic, not of molecules, but of organisms. The viruses have certainly provided the link between the molecules of the chemist

respect to structure, ranging from the smaller viruses, which are simple nucleoproteins with many properties similar to those of ordinary molecules, on through viruses with a gradually increasing complexity of structure, to the larger viruses, which, with respect to structure and properties, are similar in many respects to organisms. It must be remembered that the properties of only a relatively few purified viruses have been determined. In view of the possibility that these represent the more stable and more easily purified viruses, one cannot be certain that a true picture of the chemical and physical properties of viruses as a whole has been obtained as yet. Information regarding

most urgently. At present fission or by means of x-rays would certainly represent a most important and significant advance for the basic reaction characteristic of virus reproduction may well represent the fundamental process which characterizes all living things. A good start has been made, and the new field of virus research is ready for exploration and for development. There is good reason to suspect that the development of this field will yield information of great value to biology, chemistry, genetics, and medicine.

amount of experimental work carried out indicating that the virus activity is a specific property of the rod shaped nucleoprotein. The same nucleoprotein has been obtained from batches of mosaic diseased Turkish tobacco plants grown under different conditions and

Tobacco mosaic virus exists in the form of many strains which appear to have arisen by a process similar to that of mutation in higher organisms. Several of these strains have been obtained in purified form by means of differential centrifugation. Purified preparations obtained from plants diseased with different strains of tobacco mosaic virus were found to possess properties similar to, yet in every case distinctive from those of purified preparations of the

purified preparations of eight strains of tobacco mosaic virus has been determined. The results indicate that the mutation of a virus can be accompanied by the elimination of one or more amino acids from the virus structure, by the introduction of one or more new amino acids into the virus structure, or by a change in the concentration of one or more amino acids present in the virus structure. This work has great significance, for it has provided the first information regarding the nature of the structural changes which accompany mutation. Extension of this work may reveal the exact nature of the chemical differences between virulent and avirulent virus strains. Attempts have been made to change the structure of tobacco mosaic virus by means of known chemical reactions *in vitro* in an effort to secure chemically modified active virus. Although

crystalline nucleoprotein having individual molecules or particles about 15  $m\mu$  by 280  $m\mu$  in size, studies were undertaken in several laboratories to determine if other viruses could be obtained in purified or in crystalline form. At present, over a dozen viruses have been obtained in highly purified form, mainly by techniques involving high speed centrifugation. Some of these purified viruses are crystallizable nucleoproteins having either rodlike or spherical particles. Some are nucleoproteins which have as yet not been crystallized. Others are



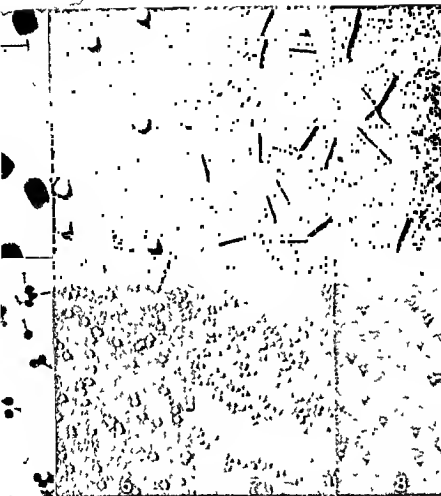


Figure 2—Electron micrographs of purified virus preparations. All are at the same magnification, and all except 1 and 5 were prepared by the gold shadow casting technique. 1, *Vaccinia* virus, 2, influenza virus (Lee strain), 3, tobacco mosaic virus prepared from hair cells, 4, potato  $\lambda$  virus (latent mosaic of potato), hair cell preparation, 5, *T<sub>2</sub>* coli bacteriophage, 6, Shope rabbit papilloma virus, 7, southern bean mosaic virus, 8, tomato bushy stunt virus (From C. A. Knight, *Symposia on Quant. Biol.*, Biological Lab., Cold Spring Harbor, New York, 12, 115-121, 1947.)

dorabies, rabies, lymphocytic choriomeningitis, and encephalitis lethargica, relatively little detailed information is available about the anatomical distribution, the character of lesions, and site of virus proliferation in many -

The mechanism of  
with vaccinia and an  
influenza, or varicella still remains obscure

In those virus diseases where the mortality is low, such as measles, mumps, sandfly fever, and benign lymphocytic choriomeningitis, the extent of permanent damage to healthy tissue following virus entry is difficult to assess, and the amount of normal cellular replacement

recovery without residual sequelae indicative of irreparable damage to nerve cells. It would thus seem that virus invasion of cells of the central nervous system is not inevitably associated with their death. There is some evidence, however, that early intra uterine infection with rubella virus may be responsible for extensive damage to the foetus and be a primary cause of many common congenital defects (Evans (1944), Swan and Tostevin (1946)).

In specific instances, invaded and apparently healthy cells may continue to function as carriers of virus, as evidenced by the liability of herpes simplex to recur at the same site on a sufferer over a period of years, and the viruses responsible for influenza and the common cold would fit into this category too. Recent work by Paul, Havens, Sabin, and Philip (1945) demonstrated that the agent or virus of serum jaundice was present in the blood of a volunteer 60 days prior to the onset of jaundice.

The phenomenon of virus interference or the ability of living and dead virus to bar the entry of live virus into a parasitized cell has attracted much attention of late. Magrassi (1937), originally re-

neurotropic and pantropic yellow fever virus in injected monkeys. Later Findlay and MacCallum (1937) showed that Rift Valley fever virus protected monkeys against strains of pantropic yellow fever virus. Similar mutual incompatibility was revealed with influenza viruses A and B, when cultivated in the embryonated egg (Henle and Henle 1944, and Ziegler, Lavin, and Horsfall, 1944). Likewise

beig (1940) connection with bacteriophage action (Delbruck and Luria 1942), and the plant viruses (Price, 1940)

## VIRUS AND CELL

C E VAN ROOYEN, M D, *Connaught Medical Research Laboratories,  
University of Toronto*

The earliest signs of virus action in tissue may be gross or microscopic evidence of cellular injury, with or without obvious disturbances of physiological function. Some body tissues are so frequently and constantly attacked by certain viruses that their affinity or tropism

tissue examination fail to provide a clue to the nature of the infecting organism during the life or death of the host. When present, the cytological response to virus invasions may be manifest in a multiplicity of ways which can be grouped as follows: (a) Death and degeneration of the parasitized cell, (b) recovery or development of carrier state with possible interference effects, (c) hyperplasia, and (d) tumor formation.

The affected tissues may show either intracytoplasmic or intranuclear inclusion bodies, with staining affinity for either acidophilic or basophilic dyes. Alternatively no inclusion bodies develop. In the case of viruses producing intracytoplasmic inclusions, the entire body may be composed of a clump of particulate structures referred to as elementary bodies. For example, in the human and animal pox diseases there is considerable destruction of superficial epithelium and replacement by scar tissue. In infective hepatitis and serum jaundice the work of Dible, McMichael, and Sherlock (1943) and Lucke (1944) revealed how extensive damage to hepatic parenchyma

never would be infinitely worse.

In infection of the central nervous system the tissue response may

tration. In other neurotropic infections such as the virus produced encephalitides depending on the duration of illness, there may be

state that with the exception of poliomyelitis, Borna's disease, psen

eases, and circumstantial evidence suggests that they represent the etiological agents of their respective maladies. Many are large enough to be seen in deeply stained preparations under the highest powers of the ordinary light microscope, and human diseases in which they have been found are vaccinia, variola, varicella, zoster, herpes simplex, molluscum contagiosum, trichoma, inclusion conjunctivitis and psittacosis. Also to be included in the same list are certain viruses of animals, such as fowlpox, pigeonpox, turkeypox, and canarypox, infectious myxomatosis of rabbits, and infectious ectromelia of mice. In the above mentioned conditions, the occurrence of elementary bodies is so constant as to enable their presence to be employed as a diagnostic feature. Van Rooyen and Illingworth (1944) utilized the appearance of elementary bodies in variola to constitute the basis of a simple and rapid test for identification of smallpox on the first day of rash.

virus host cell relationships have been numerous. High speed centrifuge design has resulted in the evolution of angle head and Sharples bowl types suitable for virus studies with rotational speeds ranging up to 60 000 revolutions per minute and gravitational forces up to 250,000  $g$ . Analytical type instruments fitted with optical devices for recording the sedimentation rate in the centrifugal field have played a conspicuous part. Likewise Tiselius electrophoretic apparatus has enabled observation to be made of the migration rates of purified virus suspensions in the electrical field under specified conditions.

Such physical methods have provided criteria respecting the sedimentation rate, electrophoretic behavior, size, shape, density, and degree of homogeneity of suspensions of virus particles. According to these standards papilloma virus approximates to a pure nucleoprotein. Unfortunately, attempts to purify many of the viruses pathogenic to man have been less successful, possibly with the exception of influenza virus. One worker, Kabat (1946), has even questioned the value of analytical data so far produced as indices of purity of the animal viruses merely.

At present it is not possible to generalize or draw any conclusions about the behavior of animal viruses. Each group of viruses has its own individual biological and biochemical characteristics. Thanks to the work of Stanley (1935), Bawden, and others the plant viruses are now regarded as specific. Some of the animal viruses, however, are phospholipid, neutral

and some are not. The growth of these particles may proceed by a method of binary fission in a manner

To summarize, it may be said that there exist many well recognized examples of virus interference phenomena and that the theoretical basis and practical applications of these reactions are worthy of further exploration.

Perhaps the most interesting of all virus action is the ability of certain mammalian, avian, and amphibian viruses to start cell division. We are all familiar with the histological character of simple superficial epithelial proliferation observed in the human wart. More brisk tissue reaction followed by hyperplasia, papillomatous formation, and, occasionally tumour production are known to follow infection in the case of the rabbit papilloma virus (Shope and Hurst, 1933), and even anaplastic squamous cell carcinomas have been reported to originate in infected rabbits (Smith, Kidd, and Rous 1947). The behaviour of the Rous (1911) avian sarcoma producing virus is too well known to merit further description, and the filtrable virus

in vaccinia and believed them to be spores of micrococci.

During the subsequent years, many bacteriologists, influenced by morphological resemblances to bacteria, have felt that the larger animal viruses were degraded micro organisms which had lost their form and metabolic independence by virtue of prolonged intracellular habitat and extreme parasitic adaptation to individual tissue cells. The late R. G. Green (1935) propounded the attractive theory that

all growth factors. Laidlaw (1939) also referred to viruses living "a borrowed life truly the supreme summit of parasitism" and dependent on the invaded cell for provision of enzymatic activity requisite for multiplication. The term "autocatalytic proteins" has also been applied by Northrup. Others have alluded to the similarity of viruses to genes. It has been pointed out that both are large nucleoproteins suggestive of self replicating cytoplasmic constituents possessing affinity for particular cells. Even the relatively large virus of vaccinia still remains unclassified, for as Beard (1948) has commented, few workers have led that

any serious thought to the matter or comprehended the importance of solving this outstanding biological problem. Elementary bodies are frequently associated with infective tissue in a number of virus dis-



A number of viruses have been shown to possess distinctive formation. The early studies of Green, Anderson, and Smadel (1942) showed that vaccinia virus was brick shaped, containing internal structure within a limiting membrane. According to Dawson and McFarlane (1948), increase of salt concentration at low temperature results in slow flocculation of virus, and redispersion by sonic vibration is possible without loss of viability. The virus could be digested with pepsin and all the phosphorous and desoxyribo nucleic acid of salt extracted virus retained its structure. Ribonuclease liberated soluble structure of the residual cell.

to consider the existence of an envelope containing a central dense core. It was capable of

is elementary

In a speci

men of variola fluid submitted to me by Dr E S Horgan, of the

of accurate measurements by Boswell (1947) has shown that the virus of molluscum contagiosum measured  $320\text{ m}\mu$  by  $226\text{ m}\mu$  with maximum deviation of  $\pm 15$  percent and the results expressed graphically in the form of a frequency distribution curve. Fowlpox virus measured  $332\text{ m}\mu$  by  $264\text{ m}\mu$  with deviation of 28 percent and ectromelia measured  $300\text{ m}\mu$  by  $210\text{ m}\mu$ . A photograph of a complete Marchal body has also

interesting aspects of virus research and trust that this paper has stimulated your interest in this new and expanding branch of microbiology.

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analogous to that of bacteria. On the other hand it is also possible

provide a solution to the problem of virus growth.

The need for intensified research into normal cell metabolism, coupled with inquiry into that of the chemical composition and biochemical activity of virus protein, is not only of academic importance

among the thousands of other chemical compounds which have been tried, little but minor effects have ensued. Cutting et al (1947) concluded that virus infections appear to be influenced by deficiencies, excesses, or alterations in their intracellular substrates. Work along these lines has been reported by Green, Rasmussen, and Smadel (1946), who showed that nitroalkridine inhibited the multiplication of influenza virus in the tissues of the developing chicken embryo, and McClelland and van Rooyen (1948) found similar results with the aromatic amidine named hexamidine. Both substances were inactive against experimentally induced influenza infection of mice, and this furnished yet another indication of the highly specialized nature of virus host cell parasitism.

The intracellular habitat of viruses calls for their study, physical and biochemical, within parasitized cells, and I should like to refer to some aspects of electron microscopy as directed towards fundamental and applied research in the virus field.

Electron microscopes have, for sometime, been obtainable commercially, and with certain models good resolution in the region of 10 to 20 Å is attainable. The advantages of employing high acceleration voltage have also been explored and Poole (1947) has developed a 400 kilovolt instrument utilizing the benefits of shorter wave length, decrease in average angle and spatial scattering of the electron path and great penetration, which combine to enhance contrast in dense biological specimens such as, for example, yeast cells. It is probable that still higher voltages may be employed with practical advantage, and research in the field of electron microscopy as applied to animal cytology, and improvements in the use of shadow casting, replica techniques, ultra high speed microtomes, and possibly electronic staining may reveal fresh data on the nature of virus host cell relationships.

One must, however, maintain a critical attitude and resist the temptation to draw deductions beyond what simple morphological appearances alone permit.

amino acid composition, should surely be sufficient to stimulate further work in this direction with other viruses

Now I presume that Dr Stanley does think, or perhaps I should say ear him make some  
ve developed, as to  
plants or again in

animals or in man

It seems quite clear that "variants" or "mutants" of viruses arise. I prefer the term "variant" partly because mutation is a comparatively rare event, so the geneticist will tell us, and partly because I feel that the changes which take place may not be the same as is implied by the term mutant. However, Dr Stanley would reply that the multiplication of viruses can take place so rapidly in the host that the change may still be considered as rare, but I think that he would also agree

when the virus is spreading, there is probably a continuous process of variation of the infective agent taking place, especially when the disease, and I am now thinking of the mammalian viruses, has become endemic. In these circumstances, due to development of varying degrees of resistance in the host as a result of exposure to infection, there would a-

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what is more important, is there any likelihood of maintaining some stability? This is an extremely important question from the immunologists' point of view

When a sufficient amount of purified virus has been obtained to carry out chemical examination, it may be that material from a number of different passages of the virus has been collected and that a "strain" may be a complex consisting of several variants. But, even if this were not so, when one has examined the strain chemically and correlated the data on composition with its antigenic behaviour and virulence one would like to be in a position to maintain that particular strain of virus in that particular state in which it is when the biological and chemical examinations were made. Does it appear likely apart from making a big batch of material and storing it under conditions which one hopes will not affect its virulence or antigenicity? What one would like to be able to do is to select strains with the attributes which would be the most useful in developing immunizing procedures. As far as I gather from discussions which I have had with Dr Stanley he feels like most of

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#### ABSTRACT OF DISCUSSION OF PAPERS BY STANLEY AND VAN ROOYEN

Dr I A GALLOWAY (United Kingdom), commentator We have listened to two very well prepared papers covering some of the more recent developments in virus research, and I am sure you will feel like myself that the ground has been covered so satisfactorily that no further comment is really necessary

necessary

I would like to refer in particular to the question of the chemical examination of tobacco mosaic virus strains of different degrees of virulence and in influenza to the similar chemical examination of strains of different immunological groupings, the one PR8, belonging to the A group, and the Lee strain to the B. These studies are, I

which one is up against is the question of getting sufficient material for examination. However, the fact that a difference has been shown to exist in the chemical composition of strains of tobacco mosaic virus of low and again of high grade virulence, and that two strains of influenza virus, immunologically different, have been shown, at least at the time when they were examined, to differ definitely in respect to

developments in recent years has resulted in definite information concerning the end results produced by the mutation of viruses. Dr Knight of this laboratory, through studies on the amino acid composition of purified preparations of strains of tobacco mosaic virus, has found that the mutation of a virus can be accompanied by a change in the concentration of one or more amino acids into the virus structure by the introduction of one or more new amino acids into the virus structure, or by the elimination of an amino acid from the virus structure. In one case, changes in the amounts of only two amino acids appeared to be sufficient to convert an avirulent strain into a lethal strain. There is good reason to believe that an extension of work of this type will yield additional very significant information regarding the nature of virus mutation and, in fact, perhaps information of great value in connection with the mutation of higher organisms.

At present there appears to be no likely prospect that it will be

laboratories throughout the world and advances of considerable significance may be expected during the coming years.

us that probably when your virus is spreading in plant or in animals you are getting continual changes in its attributes

I think that Dr van Rooyen covered a wide field and I am sure we are all coming to appreciate one of the aspects of his review, the virus host relationship, as a very important one. Dr Charles Nicolle was the first to stress the tendency toward an equilibrium in the association and this theme has been developed more recently by others. How important this question is becomes striking when one is attempting to estimate the potency of a virus preparation or, to even a greater extent, the potency of an immunizing agent in the case of mammalian viruses. The test animal may react in different ways according to age, sex, physiological state, environment, and probably its behaviour is influenced also by genetic factors. Even when you have considered all these possibilities you may find that any or each of these factors may exaggerate any peculiarity of the virus such as lack of invasiveness and everything is thrown out of balance. If you do not have some appreciation of how all these factors may influence the results of potency tests of one sort or another then you are not competent to interpret results.

Dr W M STANLEY (United States). Dr Galloway's questions pertain to the very heart of the virus problem, namely the nature of virus reproduction and of virus mutation. These are very fundamental problems and it would require more time than we have at our disposal to present an adequate discussion. However, I shall attempt to answer Dr Galloway's questions briefly. The first question with respect to the manner in which viruses reproduce, cannot, of course, be answered at the present time. However, there are two modes which might be considered. We are all familiar with the usual method of reproduction by means of the division of cells and there is a possibility that a similar process may be employed by some of the larger viruses. However, certain difficulties are encountered when one attempts to apply this process to the duplication of some of the smaller viruses which are crystallizable nucleoproteins. However, chemists are quite familiar with synthetic processes, especially those involving enzymatic processes, and they are quite willing to consider some new order of this type of synthesis as the basis for virus reproduction. Experimental methods which are available should enable a decision as to the actual mode of duplication of viruses. For example, a test to determine whether or not any of the substance of the infecting virus particles is found in the progeny, or subsequently formed particles would provide extremely significant information.

Dr Galloway's concern over the fact that viruses appear to be changing almost continuously during reproduction is a very real one. It is a fact that most viruses do appear to change, presumably by means of mutation during reproduction. Little is known about the actual process involved in this change. However, one of the most significant

tion often removes a large fraction of the virus along with the offending organisms. Methods of choice include not only the use of bactericidal compounds but also some of the procedures currently employed in protein chemistry, whereby virus is separated from other materials and at the same time is concentrated, care being taken to avoid denaturation.

The methods discussed include those of physical separation and concentration by high speed centrifugation, those of chemical precipitation with ammonium sulfate and methanol, and in addition the direct visualization of certain viruses in suitable clinical samples by electron microscopy.

*Preparation of samples which are free from contaminating bacteria, as cerebrospinal fluid, blood, and autopsy tissues removed soon after death*—Such material should be collected under sterile precautions, placed in sterile containers, kept cold, and inoculated as soon as possible into test animals. Solid tissues are often frozen on dry ice

buffer, and then centrifuged lightly to remove the abrasive and gross particles.

With such bacteriologically sterile specimens, the problem is relatively simple, for the material may then be inoculated directly into the appropriate host with no further treatment. Where the amount of virus in the sample is apt to be low, advantage may be taken of the size of virus particles, in that the virus in such specimens may be concentrated by ultracentrifugation.

certain virus strains in laboratory hosts. Thus, following the primary isolation of certain strains of poliomyelitis virus in the monkey, it is not always easy to obtain a second successful transfer in this experiment.

These pellets are taken up in 1 cubic centimeter of water or a buffer and inoculated intracerebrally. On occasion it has been possible to obtain successful passages of strains only after concentration in this fraction (2). It should be mentioned in passing that these pellets are not composed only of virus particles for if suspensions of normal tissue are treated in the same manner, pellets of a similar nature are obtained.

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# CHEMICAL AND PHYSICAL METHODS FOR THE PREPARATION OF CLINICAL SAMPLES FOR VIRUS STUDY<sup>1</sup>

JOSEPH L. MELNICK, *Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn*

This paper attempts to review and illustrate certain of the newer laboratory methods which have been developed and used for the diagnosis of both clinical and experimental virus diseases. For such purposes, the inoculation of animals and incubating eggs still re-

main the most important methods in test animals, it deals only with the preparation of clinical and other materials for animal inoculation. As examples I shall cite briefly those techniques in use in the Yale Polio myelitis Laboratory because they serve to illustrate the type of approach to this problem and because of my greater familiarity with them.

Although tissues obtained at autopsy may sometimes be used in the

tick fever and Russian spring summer encephalitis; (3) phlebotomus flies in sandfly fever; (4) nonbiting flies in poliomyelitis; (5) birds,

specimens can be inoculated into test animals. Experimental evidence indicates that all viruses consist, at least in part, of biologically active proteins. Needless to say, it is of the utmost importance that the methods used to remove bacteria (and other toxic substances which may be present) do not also destroy the virus in the sample. The older method of filtration through Berkefeld and Chamberland filters is no longer necessary, which has its advantages in that filtra-

<sup>1</sup> Aided by a grant from the National Foundation for Infantile Paralysis.



isolating virus, however, are improved if the virus in the sample is concentrated and refined, and more sensitive routes of inoculation are employed (16). Thus the crude suspension may be spun either in a refrigerated Sharples centrifuge (2 inch cylindrical rotor) at approximately 25,000 revolutions per minute for 20 minutes, or in the International PR-1 refrigerated centrifuge (6 inch rotor) at 18,000 revolutions per minute for 20 minutes. About 10 cubic centimeters of the supernatant fluid is removed, shaken with 5 cubic centimeters of ether, and placed in the ice box overnight. The following day the ether is removed, and the aqueous extract is inoculated intraperitoneally. Essentially this procedure in conjunction with intranasal inoculation of crude material has been employed by several workers (9, 17).

An even more sensitive method (16) carries the preparation of the sample further, as follows. The remaining supernatant fluid is spun in the ultracentrifuge (6 inch rotor) at 39,000 revolutions per minute for 60 minutes. This supernatant fluid is discarded, for it is devoid of all but traces of virus but still contains almost all the small molecular compounds, which often include material of toxic nature. The virus containing pellets are resuspended in 2 to 3 cubic centimeters of heated 10 percent normal monkey serum and treated with 1 to 2 cubic centimeters of ether overnight in the ice box. The ether is then removed, and the suspension spun at 18,000 revolutions per minute for 20 minutes in the refrigerated International centrifuge. To the supernatant fluid there may be added 0.05 cubic centimeter of penicillin (500 units) and 0.05 cubic centimeter of streptomycin (5 milligrams) per cubic centimeter, and 1 cubic centimeter is then inoculated intracerebrally into a monkey.

As illustrated in figure 1, it is possible of course to obtain positive isolations of virus with material administered by only one route but certain routes seem to allow smaller amounts of virus to be detected than others. The most sensitive route of inoculation for neu

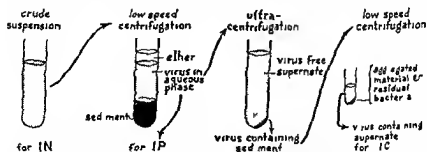


Figure 1.—Preparation of heavily contaminated material such as stools or insects for inoculation into monkeys in testing for poliomyelitis virus. IN, intranasal instillation, IP, intraperitoneal inoculation, IC, intracerebral inoculation.

fluid (2) However, the latter virus may be completely thrown down if the speed is increased to 32,000 revolutions per minute for 60 minutes

*Use of antibiotics*—Chemotherapeutic agents like the sulfonamide drugs, and especially penicillin and streptomycin, are particularly useful in samples contaminated with sensitive bacteria and (a) where the clinical samples are small and concentration is not feasible, or (b) where the amount of virus is present in sufficient amount to infect without concentration These agents are commonly used with chick embryos in isolating influenza virus from pharyngeal washings (3), or mumps virus from saliva (4, 5) Before adding antibiotics to a bacterially contaminated sample, one should be certain that the antibiotic has no effect on the virus In particular, some of the large viruses seem to be inhibited by these agents (viz, lymphogranuloma venereum by sulfadiazine (6) and psittacosis by penicillin (7) and chloromycetin (8))

Ether, which is bactericidal but does not adversely affect such viruses as poliomyelitis (9), vaccinia (10), ectromelia (11), and apparently hepatitis (12), is also useful for removing bacteria from such ether resistant viruses In poliomyelitis laboratories, stool extracts (9), pharyngeal materials (13), and insect suspensions (14) may be rendered fit for animal inoculation by contact with 15 percent ether for about 16 hours in the refrigerator Care must be taken to remove the ether from the aqueous phase containing the virus before the latter may be safely inoculated into animals, especially if the material is to be inoculated into the brain This is conveniently done by aeration or evacuation In addition to the use of ether and antibiotics alone or in combination, it is often wise to employ these compounds as a step in the preparation of larger samples of heavily contaminated materials

*Preparation of heavily contaminated and toxic samples, as stools and flies*—The procedures described here are those used in the Yale Poliomyelitis Laboratory They illustrate how certain procedures

are used in some species of monkeys (notably *Macaca cynomolgus*), which appear to have a somewhat greater susceptibility to oral and other peripheral routes of administration

A sample of about 30 to 50 grams is desirable This is mixed with two volumes of cold sterile distilled water (or M/50 phosphate buffer at pH 8) in a cold Waring mixer for 5 minutes The resultant thick mixture is suitable for intranasal inoculation (15) Chances for

*Methanol precipitation*—Cox and his associates (23) have extended to the virus field the work of Cohn on the fractionation of blood proteins by low temperature alcohol precipitation. They have shown that influenza virus may be concentrated and partially purified by this treatment. We have found also that when subjected to one cycle of methanol treatment the Lansing strain of poliomyelitis virus in murine CNS may be completely recovered under appropriate conditions of pH, namely, precipitation at pH 6 and elution at pH 7.5. But a second cycle of methanol precipitation in our hands destroys a large part of the virus. We have used this method on stools from poliomyelitis cases and found that such alcohol precipitates may contain virus, but we are not yet prepared to state how efficient the method is in permitting good recovery of virus from this source.

Experiments to determine effects of the medium in which the virus is present have been carried out (24). Whereas the bulk of normal brain proteins is precipitated at pH 6, the maximum precipitation is obtained at about pH 4.5 in stool extracts. When poliomyelitis virus (Lansing strain) was diluted in one instance with normal central nervous system suspension and in the other with normal human stool suspension, tests for virus recovery showed that the virus was found associated with each of the fractions precipitated by methanol from brain or stool. Thus, virus was found distributed throughout the three stool fractions obtained at pH 7, pH 6, and pH 4.5. When precipitated from a brain suspension, virus was found only in the pH 7 and pH 6 precipitates and not in the pH 4.5 fraction, which in brain is almost negligible in amount.

*Electron microscope*—Although this instrument has been used extensively in morphological studies of viruses, it is only beginning to be applied as a diagnostic tool. If a virus has a characteristic morphology, as in the case of vaccinia, then it may be easily recognized. Thus it

strate viruses of variola and vaccinia recovered directly from lesions of smallpox and generalized vaccinia in man. Poxlike elementary bodies were also obtained by these workers (26) and also in our laboratory (27) from vesicle fluid from cases of varicella, when such material was examined directly in the electron microscope.

We have also been able to show with this instrument that in the viremia encountered in certain animals in experimental vaccinia, the virus is present almost exclusively in the white cell fraction of the blood (28), a finding which Smith (29) reported 20 years ago on the basis of infectivity tests. The application of this instrument to clinical

otropic viruses in general is the intracerebral one, and the data indicate that the intracerebral route is favored in poliomyelitis also (16)

The choice of strain or of species of laboratory animal used in the attempted virus isolation must be considered, although data on this subject are woefully lacking. Thus, if attempts are made to isolate poliomyelitis virus from flies, there appears to be some advantage in using the cynomolgus over other species of monkeys (14, 18)

*Preparation of large volumes of heavily contaminated samples, such as sewage*—The problem here is to concentrate the virus and simultaneously to eliminate bacterial and other toxic matter. The following procedures have been used for detecting poliomyelitis virus in naturally contaminated sewage, and may serve for purposes of illustration

(a) If the sewage contains adequate amounts of virus, it may simply be etherized and inoculated intraperitoneally into monkeys. About 25 cubic centimeters of sewage may be safely inoculated into one monkey (19, 20)

(b) The virus in large amounts of sewage may be inoculated after it is concentrated, and for this the method of Gard has been useful (21). Four hundred cubic centimeters of the sample are treated with 10 cubic centimeters of ether, corked, and placed in the refrigerator overnight. The next morning the ether layer is removed and discarded. To the aqueous phase, 6 cubic centimeters of normal monkey or horse serum (inactivated at 56° C for 2 hours) are added, followed by 160 grams of ammonium sulfate, with stirring until the salt completely dissolves. After centrifugation at 2,000 revolutions per

are inoculated intraperitoneally into a monkey

(c) A more sensitive method allows one to use even larger amounts of sewage and, perhaps even more important, permits the use of the sensitive intracerebral route for inoculation (22). Eight hundred cubic centimeters of the sample are treated with ammonium sulfate as outlined, using double quantities of the reagents. The dialyzate is centrifuged in the cold at 4,000 revolutions per minute for 20 minutes. The sediment is discarded, and the supernate subjected to ultracentrifugation at 39,000 revolutions per minute for 60 minutes. This supernate is discarded and the gelatinous sediment taken up in 1 to 2

## Session 2. THE RICKETTSIAL DISEASES

Tuesday, May 11, 9:30 a. m. to 12:00 m.

Auditorium of National Museum

### ILEA-BORNE AND LOUSE-BORNE TYPHUS IN MEXICO

M. RUIZ CASTAÑEDA, *The Typhus Laboratory of Mexico, Hospital General, México, D. F.*

The concept of the etiology of typhus has undergone interesting changes in Mexico. During the last century and even more recently, there were clinicians who suspected that the Mexican disease was different from the one known in the United States. With the advent of experimental methods and the available literature between Mexican and American typhus, when the work of Neil might have become the first argument against the prevailing unitarian doctrine. But its significance was not realized until the work of Mooser 10 years later. New fields were opened to investigation, and among others, Maxcy, Zinsser, and Dyer undertook studies of considerable importance.

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of the virus which caused typhus in certain zones of the United States, a suspicion that was confirmed by Dyer in finding infected rat fleas and by Mooser, Castañeda, and Zinsser in the isolation of the agent from the brains of rats.

### EPIDEMIOLOGICAL ASPECTS

The frequency with which murine strains were isolated from patients during the years of Mooser's early investigations in Mexico resulted in the view that "Tabardillo," or Mexican typhus, was entirely murine, although a few strains of classic like typhus were found

## SUMMARY

A brief survey of some physical and chemical procedures used in a clinical virus laboratory is presented. Although each virus must be

offending bacteria and other toxic substances and at the same time concentrate the virus without denaturation so that chances of its producing infection in laboratory animals are increased.

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selective agglutination confirm these findings, not only with sera

under way may throw more light on the matter. However, in 1931 *Mus rattus rattus* was relatively common in old buildings, where it

typhus strains isolated from the brains of rats were found in black rats captured in an old prison in Mexico City. Since then the virus has been found on relatively few occasions in Norwegian rats.

The assumption that in Mexico City the decrease in human cases of murine typhus is a reflection of the low index of flea infestation of rats seems supported by the evidence that in the rest of the country rat fleas are abundant and the chances of human infection therefore greater.

Although we have little information on the actual status of typhus in rodents, we suspect that rats continue to be a potential danger in Mexico City as elsewhere, since the flea is not the only vector from rat to rat and since other species of fleas not specially adapted to rats or mice may feed on these animals and eventually transmit infection to man.

The epidemiologic features of murine typhus herein discussed have a bearing on the selection of adequate measures for the protection of man. It appears to us that the danger from this type of infection does not rest entirely on the incidence of infected rodents or even on the index of flea infestation, but that the index of human louse infestation is of major importance. We were impressed by the opinion of Mooser who believed that the epidemic occurring on the Mexican American border in 1917, when Neil isolated the first orchitic strains, was louse borne murine typhus, and there is no doubt that Mooser's cases studied in 1927-28 were also louse born typhus. In recent years Silva, in our laboratory, and Leon have reported studies on outbreaks of murine typhus in which lice were found infected and constituted the most probable vector.

#### DISTRIBUTION

in other regions of the country. Because of transient adaptation of some of these strains, with the production of the orchitic reaction characteristic of murine typhus, Nicolle suggested the existence of intermediate strains, a suggestion that encouraged investigators in

typhus, as a definite entity, has been under study lacks sustaining evidence. The fact that it has been possible in our laboratory to produce simultaneous infections, with murine and classic rickettsiae, to

cycle may change to one of man louse man. The role of lice in the transmission of murine typhus was shown by Mooser and has been frequently corroborated by the finding of the naturally infected in

type. This assumption is based on the belief that all of the strains isolated from patients during the years prior to the Mexican Revolution were identical with those described by Nicolle. At the present

times of considerable importance have not been observed during the last 15 years.

Mexico. At the present time, however, murine typhus seems to be relatively unimportant in its incidence and severity in comparison with classic typhus. Some local forms —



selective agglutination confirm these findings, not only with sera collected during the last few years but also with material saved from patients studied since 1937

value because the surveys were not extensive. Rat campaigns now under way may throw more light on the matter. However, in 1931 *Mus rattus rattus* was relatively common in old buildings, where it nested in dry places above ground, thus providing an ample harborage for fleas. Today this species is practically extinct, and the prevailing Norwegian rat that nests in damp locations underground has not been found conspicuously infested by fleas. It may be recalled that the first typhus strains isolated from the brains of rats were found in black rats captured in an old prison in Mexico City. Since then the virus has been found on relatively few occasions in Norwegian rats.

The assumption that in Mexico City the decrease in human cases of murine typhus is a reflection of the low index of flea infestation of rats seems supported by the evidence that in the rest of the country rat fleas are abundant and the chances of human infection therefore greater.

Although we have little information on the actual status of typhus in rodents, we suspect that rats continue to be a potential danger in Mexico City as elsewhere, since the flea is not the only vector from rat to rat and since other species of fleas not specially adapted to rats or mice may feed on these animals and eventually transmit infection to man.

The epidemiologic features of murine typhus herein discussed have a bearing on the selection of adequate measures for the protection of man. It appears to us that the danger from this type of infection does not rest entirely on the incidence of infected rodents or even on the index of flea infestation, but that the index of human louse infestation is of major importance. We were impressed by the opinion of Mooser  
the Mexican American

in 1927-'28 were also louse born typhus. In recent years Orr, laboratory, and Leon have reported studies on outbreaks of murine typhus in which lice were found infected and constituted the most probable vector.

### DISTRIBUTION

The geographical distribution of typhus in Mexico has been tentatively presented by Varela based on the classification of the strains

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factors influencing the epidemiology of the disease. It is obvious that the climatic conditions of each zone constitute the most important fac

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The true incidence of typhus in the tropics is not known, but in certain zones of extensive agricultural development in which medical care of the population is under Government supervision, human cases are not infrequently reported. Strikingly, a number of surveys in the Northern States of Mexico have shown a high percentage of rodents, with positive complement fixation for murine antigens. However, the incidence of human cases is rather low.

..

human cases of murine typhus have been frequently observed, at the present time the classic type is the prevalent form in these zones.

#### SKIN TEST FOR EPIDEMIOLOGICAL SURVEYS

We have investigated the possible usefulness of skin tests for epidemiological surveys. The test is based on the fact that nearly 100 percent of individuals with a history of past typhus infection have shown a delayed skin reaction at the site of the intradermal injection of a detoxified suspension of either murine or classic rickettsiae. On the other hand, the test has been negative in persons living in regions where neither flea borne nor louse-borne typhus has been reported. The skin reaction appears to be the

On large groups of individuals lacking a positive history of past infection, many develop reactions identical with those observed in immune persons. The investigations have been conducted mostly in schools, hospitals, and prisons, with a

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selective agglutination confirm these findings, not only with sera collected during the last few years but also with material saved from patients studied since 1937

These changes in the prevalence of one or the other type are difficult to interpret. A possible explanation is the changing rat population in the city, although the data on which this concept is based are of limited value because the surveys were not extensive. Rat campaigns now under way may throw more light on the matter. However, in 1931 *Mus rattus rattus* was relatively common in old buildings, where it nested in dry places above ground, thus providing an ample harborage for fleas. Today this species is practically extinct, and the prevailing Norwegian rat that nests in damp locations underground has not been found conspicuously infested by fleas. It may be recalled that the first typhus strains isolated from the brains of rats were found in black rats captured in an old prison in Mexico City. Since then the virus has been found on relatively few occasions in Norwegian rats.

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factors influencing the epidemiology of the disease. It is obvious that the climatic conditions of each zone constitute the most important factor in the prevalence of either type of infection and in the setting for the transmission of murine typhus to man. In tropical and subtropical climates, where the human louse is rare, murine flea borne typhus is

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d on large groups of individuals lacking a positive history of past infection, many develop reactions identical with those observed in immune persons. The investigations have been conducted mostly in schools, hospitals, and prisons, with a

certain endemic zones giving approximately 15 percent positive with adults.

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parent in children. Moreover, laboratory workers whose childhood

had been spent in endemic districts have been the only exception to accidental infection. The planation of the high skin test surveys

We believe that for practical purposes the skin test could be utilized to determine the index of immunity, if not with exactness the typhus incidence

### MEASURES AGAINST TYPHUS

Because of the factors herein discussed, the control of typhus in Mexico offers considerable difficulties. The public health authorities have enforced regulations based on experience acquired in successful campaigns elsewhere, but relatively little has been achieved except in zones where sanitary measures have been aided by improved standards of living. In view of this complicated situation, we have advocated that measures adopted should depend on the prevailing epidemiologic aspect of the disease in each zone rather than the incomplete application of wholesale campaigns inspired in those conducted by the Allies during the last war.

As already indicated, murine typhus should be considered under two different aspects, one of which requires handling like classic typhus. Both types offer promise of control by delousing campaigns, namely, by the use of DDT. Outbreaks of louse borne typhus are readily confined to limited areas, and the benefit of these measures has been particularly important in endemic zones of dense population.

As a prophylactic measure, the use of insecticides has been found effective wherever a permanent campaign is maintained, but unfortunately this measure requires personnel and equipment not available in the mountainous country.

the mountainous country

The urge for the use of insecticides is as intense today as was that for soap and water in the past. The latter failed because for many people soap and water were not always available. And the urge for the use of insecticides may fail because of a similar lack. We believe that improvement in standards of living and education of coming generations will do more to control typhus than one can expect to achieve with the present recommendations under existing conditions.

Ever since rats became associated with human communities, everywhere, with results that even now are murine typhus was added to the such rodents are potential carriers. rat campaigns have been encouraged, and in some countries are of considerable importance. In Mexico there has been an increasing interest in the extermination of rodents, which are, as everywhere,

factors influencing the epidemiology of the disease. It is obvious that the climatic conditions of each zone constitute the most important fac

a typical strain was isolated

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#### SKIN TEST FOR EPIDEMIOLOGICAL SURVEYS

where neither flea borne nor louse borne typhus has been reported over a long period. The nature of the skin reaction appears to be the same as those of bacterial allergies.

When the skin test is performed on large groups of individuals lacking a positive history of past infection, many develop reactions identical with those observed in immune persons. The investigations have been conducted mostly in schools, hospitals, and prisons, with a total of nearly 20,000 individuals tested.

The positives in adults have ranged from 3 to 25 percent and have fulfilled the expectancy of typhus incidence in each district. In certain endemic zones giving approximately 15 percent positive with

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parent in children. Moreover, laboratory workers whose childhood

## STUDIES ON RICKETTSIAL DISEASES IN BRAZIL

J. TRAVASSOS,<sup>1</sup> *Butantan Institute, São Paulo, Brazil*

Two diseases caused by rickettsiae, Rocky Mountain spotted fever and murine typhus, have been shown to be endemic in Brazil. So far as we know, there is no reference in the medical literature to the endemic or epidemic occurrence of any other rickettsial disease in that country.

A few cases of what seems to be classic typhus, however, have long been detected by Brazilian clinicians, mainly in seaports among immigrants, who had arrived from Europe shortly before the disease. Yet no attempt to isolate *Rickettsia prowazekii* was made in any of these cases, and so their etiology could not be established with certainty.

Cases of Rocky Mountain spotted fever were first seen in 1929 in São Paulo (1). Rickettsiae were isolated from several of them. At that time, however, it was not possible to determine the exact nature of the infection which came to be known in the English American literature as São Paulo typhus. A similar disease was soon afterward shown to occur in a bordering State where it was called Minas Gerais typhus fever (2).

In the next years, an extensive investigation showed the similarity of the infections induced in laboratory animals by the São Paulo and the Bitter Root Valley strains of rickettsia. Cross immunity tests carried out by both Brazilian and American workers confirmed that the agent of the so called São Paulo typhus is a strain of *Deimacacentroxenus rickettsii* (3-8).

Shortly afterward evidence was presented that substantiated the identity of the rickettsiae isolated in São Paulo and in Minas Gerais (9-11).

Spotted fever occurs rather infrequently in São Paulo and is met with mainly in well circumscribed foci in suburban or rural areas. It is commonest in the summer and autumn months, and the peak of its incidence is usually reached in November. A few cases may occur in every month of the year throughout the State. In certain localities of its affect Negr eral the same name (12-14).

Clinically, the disease resembles the severe type of Rocky Mountain spotted fever prevalent in the Bitter Root Valley (15). It is usually characterized by abrupt onset, severe prostration, high temperature

<sup>1</sup> Read in the author's absence by Dr. N. H. Topping.

the cause of considerable economic loss. These campaigns have been welcomed by all and are expected to become more and more important in the near future. The extermination of rodents would be of particular benefit in regions where murine typhus may become house borne and also in zones where flea borne typhus is frequently transmitted to man. The modern methods for the destruction of rodents, together with the encouraging results of their application in the United States, have stimulated great interest in rat campaigns in Mexico. Personally, I consider it most fortunate that large scale work of this type is possible, but as an orthodox typhus student I believe that if such campaigns are emphasized as the most important measure against typhus, that may mislead the sanitary effort in countries like Mexico where classic typhus continues to be the major problem. The destruction of rodents needs no defense. They are bad enough for reasons other than typhus. But a logical use of

duce the density of the endemic infection, and even with deficient individual protection the mortality would be reduced to a minimum.

Recent studies on the protective value of typhus vaccines have been made in Colombia by Montoya, who found that the vaccines used had a high protective effect in man. Eight thousand individuals were vaccinated, half of them with Cox's and half with lung vaccine. Only 2 became infected in the vaccinated group, while in a similar number of controls over 30 cases of typhus were observed.

The choice of vaccine is of little importance provided the one chosen contains sufficient amounts of rickettsiae. The production of purified suspensions of the organisms by our lung method, we have found to be practical. We have found that a mixture of classic and murine antigen constitutes an excellent material for the protection of man, one that reduces the incidence of infection and reduces the severity of the disease if infection occurs. Therefore we have been using vaccines prepared in this way for human immunization in Mexico.

work has limited the us, but we hope that e profit, especially in zones where delousing campaigns cannot be maintained as a permanent practice.



ment-fixation test. A standard serum prepared in the National Institute of Health with a Bitter Root Valley strain and several sera prepared in Sao Paulo with a local strain were tested with two antigens, each prepared with one of these same strains. The titer of each serum was the same regardless of antigen used.

Evidence to the same effect is provided by the results of xenodiagnostics and the observation, similar to the one first reported by Munter (30), that the inoculation of the São Paulo virus fails to induce a renewed production of Proteus X agglutinins in rabbits previously injected with a Bitter Root Valley strain, and vice versa (9).

Epidemiological data support the claim that infection is contracted by man through the bite of blood sucking insects around rural or semirural dwellings (12, 15, 16, 17).

The occurrence, simultaneous or otherwise, of multiple cases of infection in the same home does not seem to favor the theory of a man to man transmission of the disease, either directly or through lice, fleas, or bedbugs (12). Moreover, several laboratory experiments provide evidence against the epidemiological importance of such arthropods (31, 32). There are reports about isolation of the virus from lice and bedbugs (33, 2), but these most likely had been caught soon after feeding on spotted fever patients.

Some observations and experiments contribute to establish the theory of transmission through the bite of the tick on what seems to be a solid basis. In some cases, ticks were caught while still stuck into the body of a person suffering from spotted fever (15). In other instances, the patient reported that he had been bitten by a tick some days before feeling ill.

Many naturally infected *Amblyomma cajannense* (18, 34) and *A. striatum* (35, 36) were caught on dogs. The infection was transmitted to guinea pigs (36), by allowing the tick to feed on them or

infected animals naturally have been reported  
fever

(a) *Amblyomma cajannense*. In adult stage, on the ground, on horses, donkeys, dogs, hares, wild rabbits (*Sylvilagus munensis*), opossums, and on human patients with spotted fever, as nymph, on horses, opossums (*Didelphis aurita*), and, before feeding, on the ground, as larva, again on the ground, before feeding.

(b) *A. striatum*. In adult stage, on dogs, as nymph, on body of a child who was forthwith vaccinated and escaped infection.

(c) *A. cooperi*. In adult stage, on the big Brazilian rodent *Hydrochoerus capybara*.

(d) *Ixodes loricatus*. In adult stage, on the opossum (*Didelphis paraguayensis*).

rash frequently coming out as early as the third day of fever, and marked tendency to hemorrhage and skin necrosis. Mortality is high, usually around 70 percent, but it may reach 90 percent, and even more in some restricted areas (12, 15-18)

The Weil Felix reaction is frequently positive with the Proteus X19, X2, and XK, the titer of the first being in almost every case the highest one. In some rare instances, the X2 titer exceeds the other two. The test is negative in about 50 percent of the cases that end in death within the first week of fever (15, 19)

Felix's view (20, 21) that a special antigenic type of Proteus X, namely, OX1<sub>L</sub> (22), corresponds more closely than either OX19 or OX2 to the São Paulo strains of *Dermacentrozetes rickettsi* was not confirmed experimentally (23)

Work by the author shows the usefulness of the complement fixation

that run a rapid and fatal course

the drop of the temperature below normal. Of these, only a few monkeys and guinea pigs survive (15, 24)

The mortality in guinea pigs keeps usually around 70 percent, but it may exceed 90 percent with some strains. It is much lower in rabbits

The inoculation of the São Paulo virus into the mouse, rat, and opossum produces a symptomless infection which rarely if ever ends in death (24-27)

In susceptible animals, the infection usually brings about an increase in size of the liver and spleen. The latter is sometimes considerably enlarged, looks darker than normally, and is covered with a small amount of exudate.

Less frequently (about 25 percent of the inoculated guinea pigs) the rickettsia gives rise to an inflammatory reaction with swelling and hemorrhage of the scrotum, which may go on to necrosis (15,

Guinea pigs can sometimes be protected against inoculation of virus by the injection of homologous immune serum (4, 15, 28)

The identity of the São Paulo and the Butter Root Valley strains was once more confirmed in our laboratory by means of the couple

white and the gray mouse, are very little susceptible to the virus and usually develop a symptomless infection after being inoculated (24, 25). When injected in series from rat to rat, the virus usually disappears after 2 or 3 passages (19).

into the rats (30). We did not, however, have to employ such a technical device to keep the virus without loss of virulence in the organism of the wild rat *Aecomys squamipes* through 10 passages in series (51).

• The dog is probably another natural reservoir of the Sao Paulo virus (13, 14, 31, 52). Although *Dermacentor zenois rickettsi* has not so far been isolated from the dog, there is evidence that the virus may

*Glyomma cajennense* and *A. striatum*) have been caught on dogs (14, 34, 35, 36). Proteus X19 agglutinins, occasionally at a high titer, have been detected in dog blood (13, 14, 52). A number of the dogs that gave a positive reaction lived in some focus of spotted fever and others were found carrying infected ticks.

When injected into the dog, the Sao Paulo virus does not give rise to an apparent infection. Yet there is evidence that it may remain viable for some days after the inoculation (53).

Ticks (*A. cajennense* and *A. striatum*) have been infected after being permitted to feed on domestic and wild dogs at any time from the fourth to the ninth, and exceptionally to the eleventh day after inoculation of the virus (53). More evidence that the dog may play a role in the spreading of spotted fever in Sao Paulo is afforded by an observation not reported in the literature. In a house where there was a case of the disease, infected ticks were found on a dog that had lived until some days previously in a focus of São Paulo typhus miles away. There are several other reports of similar isolated cases, the occurrence of which might be explained in a like manner. Thus the dog, as carrier of ticks, whether infected or not, seems to have an important part in the epidemiology of spotted fever in Sao Paulo.

The prophylactic measures for the campaign against the Sao Paulo typhus had two main aims: (a) Eradication of ticks, for which general and personal rules have been advised, and (b) immunization of humans in the foci of infection by means of vaccination and periodical revaccination (54).

It is not that I have been scarce, if any  
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ensive methods

involved

Since 1938 vaccination and revaccination have been carried out regularly in the main foci of infection. For several years a Spencer

\* Several samples of the other ticks were caught and permitted to feed on guinea pigs. Infection resulted repeatedly from the bite of

larvae had fed

These experiments show that the virus passes through the egg to the

ability to transmit the virus when they are infected in the laboratory. After feeding on infected laboratory animals, *Amblyomma cajennense*, *A. striatum*, *A. brasiliensis* and *A. cooperi*, as well as *Rhipicephalus sanguineus* and *Ixodes loricatus* have carried the virus into normal animals (37-42). Under laboratory conditions, *Dermacentor andersoni* seems to be an excellent vector of the so called São Paulo typhus (43).

ation to generation of the tick reservoir of the virus. The tick therefore seems to be a natural

The opossum was found naturally infected in São Paulo (*Didelphys paraguayensis* and *D. aurata*) (44) and in Minas Gerais (*D. marsupialis*) (45). Rodents were also mentioned as a possible reservoir (45).

A symptomless infection is produced by injection of the São Paulo virus into the opossum (*D. aurita*) where it remains viable for a long time. It has been passed, with a certain loss in its virulence, six times in series from opossum to opossum (27).

The Brazilian cavy (*Cavia aperea*), the wild rabbit (*Sylvilagus munensis*), and the capybara (*Hydrochoerus capybara*) (46) react to the injection of virus. Of the two first named animals, the cavy is by far the most susceptible. It is possible to transmit the virus to ticks by allowing them to feed on experimentally infected capybaras (47).

Thus far the rat (*Erymus norvegicus*) has not been found harboring the virus in nature (48, 49). The white and gray rat, as well as the



Parker type of vaccine, prepared on a large scale in the Butantan Institute, São Paulo, with *Amblyomma cajennense* infected with the São Paulo strain (54, 55, 56), has been used.

This vaccine confers on guinea pigs a high degree of resistance to virulent strains of *Derma-centrozetus rickettsi*. In addition, a Cox type of vaccine has recently been prepared for use in the vaccination and revaccination of persons in the foci of the disease (57).

A serum that showed some protective and therapeutic efficacy in guinea pigs was also prepared in the Butantan Institute (58). Penicillin and several sulfa drugs were tried out for their therapeutic action, in animal tests, without success.

Murine typhus was first recognized in Brazil in 1937. The patient was a São Paulo Health Department employee, who collected fleas

abounded

The complement fixation test was helpful in providing the only sure indication of the presence of the virus in the animals of the three first passages. Indeed, a short and slight rise of temperature was at first the only additional sign of infection.

In guinea pigs, the virus induced, from the fourth passage on, a serotal reaction which became more and more frequent and marked as the infection was transmitted in series to new animals. Smears of tunica vaginalis of infected guinea pigs frequently showed the crowding of rickettsias in endothelial cells (Moores bodies). From the fifth passage on, the Neil Moore sign was a constant feature of infection.

The identification of the virus was completed by cross-immunity tests carried out with several strains of *Derma-centrozetus rickettsi* and with the Wilmington strain of *Rickettsia moorei*. It was confirmed by the survival of the virus through numerous rat to rat passages and the results of complement fixation tests performed with a great number of sera collected from infected rats and guinea pigs. The majority of these sera gave a positive reaction when tested with a murine antigen. Consistently negative results were obtained with a Rocky Mountain spotted fever antigen sometimes employed in the tests.

Taken together, the survival of the virus through numerous rat to rat passages and the results themselves enough to

After the first cases

São Paulo Health Department began to perform systematically the Weil Felix reaction in every specimen of blood received for diagnosis of enteric fever. Many positive results with Proteus X19 were ob

occurred and remained for considerable periods of time. The washed

control material just prepared from normal eggs, we occasionally ran across people who gave skin reactions to normal egg, a point that must be considered always as a possible source of confusion when reading skin test reactions.

Prophylaxis in typhus fever is suggested by Dr Castañeda. If Dr Pidia is in the audience, I would like to ask him to discuss the

and is responsible for the reduction of typhus fever in that area. Will someone from the Communicable Disease Center in Atlanta discuss this?

To go back to Dr Travassos' paper, Rocky Mountain spotted fever is common in the United States, a few years ago, the case fatality rate is considered to be 60 to 70 percent in certain areas. I think it has been well shown now that the case fatality rates in spotted fever are dependent to a large degree upon the age of the infected individual. I feel

found to be those below the age of 15 is 12.5 percent, between the ages of 15 to 40 the rate is 13 percent, and above the age of 40 the case fatality rate is 40 percent. This same situation exists in epidemic typhus where the age of the infected individual is of considerable importance in the case fatality rate.

United States plays a part in the epidemiology but the cause is not well known. In Long Island, complement fixation tests were made on sera of dogs that live in families where spotted fever occurred. In one of these cases, the dog had an illness which preceded his master's illness by about 2 weeks, which is the usual incubation period of Rocky Mountain spotted fever.

- [illegible]

## ABSTRACT OF DISCUSSION OF PAPERS BY CASTANEDA AND TRAYLISOR

Dr NORRIS H TORRES (United States) commentator I think Dr Castañeda should be congratulated on an excellent paper. The thing that strikes me in this connection and also in connection with some of the comments on Dr Travassos' paper is that perhaps undue emphasis in the past has been laid upon the scrota reaction of the guinea pig as a means of differentiating between classical typhus and murine typhus. Perhaps Dr Snyder will mention that he isolated several strains in Madrid in 1911. The strains Dr Snyder sent us gave marked scrota reaction for some time. It is entirely possible that scrota reactions may be produced by classical strains and I think the other methods of differentiation are more reliable than the animal test. As to the skin test that Dr Castañeda mentioned, we did many tests on vaccinated individuals and also on individuals who had acquired typhus fever in the laboratory. The soluble antigen and the whole typhus vaccine gave marked skin reactions. In fact, they were so marked that in blonde individuals rather large areas of pigmentation



emético, y odorado", con resultados que han reducido el índice de mortalidad y, que al parecer han acortado el tiempo del proceso pa-

Como nota de particular importancia debo señalar lo siguiente. En Noviembre del año pasado 1947, llevó a Bolivia el Dr. E. H. Payne, el producto que se conoció como el "quinto antibiótico" o sea la "cloromicetyna". Ensayado para el tratamiento del Tifus epidémico en aplicaciones inyectadas por vía subcutánea o endovenosa y también por la vía bucal en tabletas, con el concurso local de los profesionales doctores J. Knudt y Palacios F., se le ha atribuido excelentes resultados, aunque los casos entonces tratados fueron todavía pocos.

Y efectivamente, algunos de los enfermos graves en tratamiento que observamos en el Hospital General y que seguimos el curso de la en-

Probablemente mayores pruebas con este último antibiótico con-  
vencencia

to me that the epidemiology of murine typhus shows several missing links. It has not been studied adequately. We have observed, for instance, that in rat communities murine typhus has special characteristics. We have studied typhus in two rat communities where no human murine typhus had been previously observed. When human murine typhus occurs, research has shown that murine typhus was also occurring in that place as an epizootic. For instance, in Antofagasta, in 1931, we described human and rat murine typhus, and we were able to isolate the first strain of this disease in Chile, but after the work of the antiplague  
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ther investigation

Dr. Castañeda has called attention to the disappearance of human murine typhus in recent years in Mexico. This may be due to a low infection rate in rats. Another interesting point was the discussion of the prophylactic value of insecticides and a rodent extermination campaign. This would be better than attempts to protect the human population by means of vaccination. It is not possible here to discuss this point further but I will say that in many cities in Peru and Chile, where we are conducting large campaigns against plague, we have seen the disappearance also of typhus. In the mountains of Peru, this

rather than thousands, as experienced in previous years. It is believed that the DDT dusting program has been highly effective in reducing the incidence of murine typhus by reduction of rat ectoparasites, particularly fleas.

Dr. FELIX VEINTEMILLAS (Bolivia). Están abundantemente expuestos y publicados por autores americanos y europeos los procedimientos para la profilaxia de esta rickettsiasis. Diferentes vacunas

comunicaciones que, en mi país se sufrió de fuertes brotes, aunque y pequeños en los últimos años, de la infección a rickettsia del tipo epidémico.

Se han realizado pruebas experimentales de inoculación tífica y de vacunación en animales y en humanos, así como campañas profilácticas de inmunización.

No permito aquí el límite reducido del tiempo para resumir este punto de vista que lo hemos expuesto ampliamente en nuestro libro "Tratado sobre las Rickettsiasis—El Tifus altiplánico" del año 1944 pero quiero insistir que las pruebas en el laboratorio y en el hospital fueron brillantes en sus resultados, siempre que las dosis de vacuna eran más de dos o tres, de muy alta concentración de Rickettsias y de fresca elaboración. En los seres humanos la vacuna se da en tres tipos de dosis:

animal

protegidos

Los tipos de vacunas que usamos han sido siempre a germen muerto: la suspensión de Rickettsias del tifus epidémico o murino procedente del peritoneo y de la tunic vaginal de ratas (método Zinsser Ruiz Castañeda), la suspensión de Rickettsias de origen pulmonar de roedor (método de Ruiz Castañeda) y los cultivos en embrión de pollo (método de Cox).

Hemos trabajado con productos del Laboratorio de Investigaciones Médicas de México, con productos de conocidas fábricas norteamericanas y hemos elaborado nosotros los tres tipos de vacunas para nuestros países.

Se

requiere de la revacunación después de algunos meses o de un año.

## TREATMENT OF EPIDEMIC TYPHUS WITH CHLOROMYCETIN<sup>1</sup>

EUGENE H PAINE, A M, M D, *Department of Clinical Investigation  
Parke, Davis & Co, Detroit, Mich* and JOSE A KNAUDT, M D, *Pro-  
fessor of Bacteriology, University of La Paz, La Paz, Bolivia*

This paper is a report of results obtained following the treatment of 21 cases of epidemic typhus with chloromycetin

Chloromycetin, a new antibiotic isolated by Ehrlich (1) and associates from *Streptomyces* sp, was found in soil originating near Caracas, Venezuela. It is a neutral compound stable in aqueous solution for over 24 hours at a pH range from 2 to 9. In distilled water it is unaffected by boiling for 5 hours

1947 The Province of Camacho, with a population of approximately 120,000, 90 percent of whom are pure Aymara, lies 203 km north of the capital, La Paz, on the eastern shore of Lake Titicaca, and borders Peru on the north. Puerto Acosta (population 1,200) is the seat of the provincial government. The climate is rigorous, the altitude being approximately 14,000 feet above sea level, and surrounding snowcapped mountains cause the temperature to remain low

On October 1947, the  
o Acosta  
December  
15, when the activities of Servicio Cooperativo Inter Americano de  
Sande Publica checked its progress by means of immunizations and

demic spread to other provinces where it is common

Table 1 presents the 21 cases of epidemic typhus treated with chloromycetin. The results show the rapid recovery of the patients following treatment

*Controls*—For controls we studied 50 cases of epidemic typhus occurring in the same epidemic, with the following results

Weil Felix at dilutions of 1:600 to 1:1,400, average 1:1,200

<sup>1</sup> Material used was supplied by Parke Davis & Co. Detroit Mich. The Bolivian Office of the Institute of Inter American Affairs rendered invaluable assistance in connection with this study

<sup>2</sup> Toxicity and safety tests were performed by the Research Department Parke Davis & Co. Detroit Mich

is very clear. We no longer have typhus and plague in the cities where anti plague campaigns have been carried out.

Dr M RUIZ CASTANEDA (Mexico) I wish to express my thanks for the interesting comments by Dr Topping, Dr Felix, and Dr Macchiavello, which will be most helpful in the conduct of my future work. I particularly wish to take this opportunity to pay homage to Dr Felix for his work in the serology of typhus.

No toxic reactions or signs of intolerance were observed in the dose range used. The blood count did not vary outside the limits of error for field estimation. Five normal controls who took the drug for 3 days helped confirm this observation.

### CONCLUSIONS

Chloromycetin is a safe antibiotic for intravenous use in the dosage used. Indications are that the oral dose may be increased with safety over the intravenous amounts employed.

The favorable effects of treating typhus (epidemic) with chloromycetin appear rapidly, and the patient usually enters convalescence within 3 days.

Chloromycetin is effective either parenterally or orally.

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TABLE 1—*Epidemic typhus treated with chloromycetin*

Case	Age	Sex	Well-Fells	Tem- per- ature	Pulse	Dose Daily		Total	Return to normal		Sym- ptoms relieved
						Oral	I V		Tem- per- ature	Pulse	
1	15	F	1 1200	40.9							
2	15	F	1 1200	39.7							
3	12	F	1 1400	40.6							
4	14	M	1 1200	41.6							
5	32	F	1 1200	39.0							
6	18	M	1 1400	39.8							
7	17	M	1 1200	40.0							
8	38	F	1 1200	40.2							
9	18	M	1 400	38.8							
10	45	M	1 600	( )							
11	48	F	1 1200	40.8							
12	34	F	1 600	39.7							
13	30	M	1 1200	39.4							
14	25	M	1 1200	40.3							
15	30	F	1 1200	40.1							
16	52	M	1 1200	39.6							
17	22	M	1 1200	39.7							
18	44	M	1 1200	40.2							
19	17	F	1 600	39.7							
20	43	M	1 1200	39.8							
21	64	M	1 600	39.7							

† Subnormal.

Number of deaths 14, or 28 percent.

Deaths occurred on eleventh to twentieth day of disease, average 14 days.

Patients recovering entered convalescence on twelfth to twenty-fourth day of disease, average, 18 days.

## DISCUSSION

Chloromycetin was supplied in two forms. For intravenous use,

hours after the first injection, the headache and backache showed improvement, and vision was often normal.

The solvent must be considered as a possible complicating factor in these results, but in the authors' opinion it is of minor importance.

Oral dosage was equally effective but required 8 to 12 hours longer for results to appear. Later, it was found that many of the tablets were excessively compressed and required several hours to disintegrate.

For convenience the dosage regimen adopted toward the end of the study was as follows:

studied

None of the treated group developed complications or died. One patient who received chloromycetin on the third day was discharged from the hospital for light work on the ninth day after onset. The first patient in the group was held for observation in the hospital for 28 days. The mean period of hospitalization for the group was 19.3 days.

Twenty two of the 25 treated cases derived their infections from exposures in areas within a radius of 20 miles of Kuala Lumpur.

the same spots which provided 14 patients of the treated group. Hence one may assume that the strains of rickettsiae which infected the treated and control groups were fairly comparable in virulence. The test and control groups may also be assumed to be comparable as regards capacity of the individuals to overcome infection with *R. tsutsuga*.

the controls was similar to that in the treated group. The sharp contrast in the clinical responses of the two groups is clearly evident from tabular data. The mean duration of fever in the control group was 18.1 days, two patients developed serious complications, one of which proved fatal, and the average period of time spent in the hospital was 30.7 days.

All 25 patients in the treated group received an initial oral dose of approximately 50 milligrams of chloromycetin per kilo body weight, and were subsequently given 0.2 to 0.3 gram of drug by mouth every 2 to 4 hours for a variable time. During the early part of the present work, treatment was continued until at least the twelfth day after onset, these patients received totals of 8 to 15.5 grams of drug. The duration of treatment was gradually shortened, and the last seven patients received a total of 1.5 to 2.5 grams. With this short course of therapy, no serious complications or deaths of these

treated patients have not yet been made because of technical and supply difficulties. It is of interest that chloromycetin can be employed successfully without dependence upon the results of such assay techniques. The practicality of the use of chloromycetin is further emphasized by the fact that 12 of the 25 patients were treated in estate hospitals where conditions are no more favorable for complete nursing care than in the average private home in the United States.

### CONCLUSION

Chloromycetin is highly efficacious in the treatment of patients with scrub typhus. It is simple to administer, and has not been found toxic for man.

## CHLOROMYCETIN IN THE TREATMENT OF SCRUB TYPHUS<sup>1</sup>

JOSEPH E. SMADAL, THEODORE E. WOODWARD, HERBERT L. LEY, JR.,  
CORNELIUS B. PHILIP and ROBERT TRAUB, *Army Medical Department Research and Graduate School, Commission on Immunization of the Army Epidemiological Board, Washington, D. C., and the University of Maryland School of Medicine, Baltimore, Md., and*  
R. LEWTHWAITE and S. R. SAVOOR, *Institute for Medical Research, Kuala Lumpur, Malaya*

The antibiotic chloromycetin was described in 1947 by Ehrlich and his associates. It has been shown by Smadel and Jackson to have a beneficial chemotherapeutic effect when administered to mice or embryonated eggs infected with a number of rickettsial agents or with several viruses of the psittacosis lymphogranuloma venereum group. The drug is rapidly absorbed when given by mouth to human beings, and readily reaches concentrations in the blood of the order of 40 gamma per cubic centimeter. No obvious toxic effects attributable to the drug have been observed in the normal men or the patients who have been studied to date. A preliminary note describing the encouraging results observed in a few cases of epidemic typhus who were treated with chloromycetin early this year in Mexico has been submitted by workers from the Army Medical Department Research and Graduate School and the Instituto Salubridad y Enfermedades Tropicales.

Twenty five persons with scrub typhus were treated with chloromycetin during March and April of this year. The chloromycetin used in the work was supplied by Parke Davis & Co. Each of the patients presented clinical features of the disease. Furthermore, the diagnosis was proved in each instance by recovering *Rickettsia tsutsugamushi* from the blood taken prior to treatment or by demonstrating the development of agglutinins for the OX 19 strain of *B. proteus*. Rickettsemia occurred in 20 of the 25 patients, and a positive Weil Felix in 21 of the group.

Eighteen of the treated patients were males and seven females. Their ages varied from 19 to 55 years with a mean of 33.1. Treatment was begun on the third day of illness in two instances and on the eleventh in one, the mean value for the day chloromycetin was started in the 25 patients was 6.2. The mean value for the last febrile day of illness in the treated group was 7.5. The shortest period which fever persisted after beginning treatment was 10 hours and the longest 96. The average duration of fever after the first dose of drug was 31 hours.

<sup>1</sup>Read by Col. Rufus I. Holt, Commandant of the Army Medical Department Research and Graduate School, Washington, D. C.



## LA VACCINATION DU TYPHUS ET COMMENT NOUS L'AVONS COMPRISE

PAUL GIROUD, *Chef du Service du Typhus à l'Institut Pasteur,*  
*Membre du Conseil Supérieur d'Hygiène Publique de France*

La condition humaine européenne de ces huit dernières années nous a prouvé que nous avions eu raison de ne pas abandonner l'étude des fièvres exanthématiques que nous avions commencé à Paris avec notre regretté Maître Charles Nicolle. De plus comme celui-ci nous le disait nous pensions que ces affections devaient être surtout étudiées en dehors des régions où elles se passent habituellement. Les sujets vivants dans de tels pays ne sont pas spontanément immunisés et peuvent servir de témoins absolus, leur étude sérologique est particulièrement précieuse lorsqu'on recherche les possibilités d'une immunisation véritable.

Les premiers essais que nous avons fait d'immunisation l'ont été avec des cultures desséchées de rickettsies cultivées en milieu liquide

<sup>1</sup> Mais en tant qu'élève de Ch. Nicolle nous avions appris à nous méfier des vaccinations avec antigène vivant, aussi ne nous sommes

permettre une production industrielle.

En 1937 nous avons fait des inoculations de rickettsies dans le jaune de l'oeuf près de l'embryon, mais un pourcentage élevé d'insuccès, nous avait amené à abandonner une technique qui dans les mains de H. R. Cox s'est révélée magnifique et féconde.

D'autre part la constatation de Ruiz Castañeda cultivante le virus orchitique du Mexique dans le poumon des rongeurs qui, suivant son habitude, a une fois de plus été un initiateur, nous avait semblé

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provenant du pou, avons nous collaboré avec Paul Durand pour la fabrication d'un antigène pulmonaire tant souris que lapin. Et Zinsser, auquel nous écrivions à cette époque, était heureux de notre succès mais un peu étonné d'un tel résultat avec la souche épidémique. Car il ne croyait pas bien à la sensibilité de la souris et du lapin au virus épidémique. En effet les rickettsies épidémiques cultivent localement lorsqu'elles sont inoculées dans le poumon inoculées par une autre voie cérébrale, péritonéale ou sous cutanée, il n'y a culture locale que lors du 1<sup>er</sup> passage, ainsi l'infection non associée à un autre virus ne peut être transmise que sous forme inapparente.

## ABSTRACT OF DISCUSSION OF PAPERS BY PAYNT AND SMADDEL

Col RUFUS L. HOLT (United States) I think it is a very exciting report that we have heard this morning. I think Dr Smadel and Miss Jackson are to be congratulated on the work they have done in the laboratory with chloromycetin, and I think Dr Smadel and his colleagues have made remarkably rapid progress in elucidating the effects of chloromycetin on scrub typhus. It is to be hoped that it will be used in Rocky Mountain spotted fever with equally good results.

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Les premiers essais que nous avons fait d'immunisation l'ont été avec des cultures desséchées de rickettsies cultivées en milieu liquide suivant la technique de Nigg et Landsteiner, nous avons vu qu'une seule injection intradermique ne provoquait que peu d'immunité tandis que trois injections permettaient d'acquiescer un bon résultat.

Mais en tant qu'élève de Ch. Nicolle nous avions appris à nous méfier des vaccinations avec antigène vivant; aussi ne nous sommes nous pas entêtés dans une telle voie. Zinsser, lui non plus, n'avait pas peu contribué à nous convertir à l'utilisation des antigènes tués, mais la technique de culture sur gélose aux tissus ne pouvait pas nous permettre une production industrielle.

En 1937 nous avons fait des inoculations de rickettsies dans le jaune de l'oeuf près de l'embryon, mais un pourcentage élevé d'écarts, nous avait amené à abandonner une technique qui dans les mains de H. R. Cox s'est révélée magnifique et féconde.

D'autre part la constatation de Ruiz Castañeda cultivante le virus orchitique du Mexique dans le poumon des rongeurs qui, suivant son habitude, a une fois de plus été un initiateur, nous avait semblé prodigieusement intéressante.

*Technique pulmonaire* — Aussi quand Durand et H. Sparrow eurent constaté le même résultat avec les rickettsies du typhus épidémique provenant du pou, avons nous collaboré avec Paul Durand pour la fabrication d'un antigène pulmonaire, tant souris que lapin. Et Zinsser, auquel nous écrivions à cette époque, était heureux de notre succès mais un peu étonné d'un tel résultat avec la souche épidémique. Car il ne croyait pas bien à la sensibilité de la souris et du lapin au virus épidémique. En effet les rickettsies épidémiques cultivent localement lorsqu'elles sont inoculées dans le poumon, inoculées par une autre voie cérébrale, péritonéale ou sous cutanée, il n'y a culture locale que lors du 1<sup>er</sup> passage, ainsi l'infection non associée à un autre virus ne peut être transmise que sous forme inapparente.

Mais Zinsser comprenait que, "dans une alternative comme la

animaux. Nous avons employé successivement le lapin puis, quand celui-ci nous a fait défaut le chien adulte. Le poumon de lapin ou de chien dose dans la peau donnent des résultats équivalents.

*Adaptation directe du virus épidémique au poumon sans passer par des parasites*—De plus, l'envahissement de la France et la barrière séparant Tunis et Paris, les régions à typhus, des régions saines, nous ont amené à rechercher à obtenir directement aux dépens du cobaye l'infection pulmonaire de la souris avec le virus épidémique, la technique de Weigl, nécessaire dans l'adaptation suivant Durand et Sparrow, ne nous étant pas familière. De plus, nous voulions pouvoir adapter des souches de diverses provenances, souches de l'est de l'Europe, par exemple, l'antigène que nous fabriquions étant destiné à nos prisonniers de guerre en contact avec des virus de ces régions. Cette adaptation était d'autant plus nécessaire que nous n'avions pas à notre disposition les remarquables techniques antiparasitaires que vous nous avez révélées, et nous n'avions que la vaccination comme moyen prophylactique.

Dès 1941, nous pouvions publier avec R. Panthier que les rickettsies se comportent différemment suivant l'hôte au cours de l'adaptation pulmonaire.

L'adaptation à un hôte nouveau se fait toujours de la façon suivante lorsque l'animal résiste à l'infection: on constate au lieu de la culture des rickettsies sous leur forme bacillaire des éléments arrondis de plusieurs  $\mu$  d'épaisseur ressemblant à des inclusions et que nous avons appelé en 1941 corps homogènes. Lorsque la défense de l'animal

tivement, les rickettsies ne cultivent plus que sous leur forme bacillaire. Ces constatations ont été confirmées en 1944 par Begg, Fulton et Van den Ende.

Pour la préparation de l'antigène, au début nous n'utilisions que des passages souris-lapin.

Après avoir vu avec R. Panthier que les passages ininterrompus lapin—lapin ne provoquaient pas de baisse de virulence puisque nos passages de contrôle sur souris ou cobaye se comportaient comme les passages souris-souris ou mieux qu'eux, nous n'avons plus conservé nos souches épidémiques que par voie pulmonaire sur lapin, elles sont ainsi entretenues depuis huit années.

Même actuellement, comme nous avons pu le voir avec notre collaborateur Vargues, les poumons de contrôle dosés dans la peau montrent une réaction positive au 0,01 mg. Un centième de milligramme

donne une réaction d'indice volumétrique 16 au 4<sup>e</sup> jour, 50 mg un réaction entre 500 et 600, les volumes des nodules étant exprimés par la formule  $r^2 h$   $r$  étant le rayon moyen du nodule en millimètre et  $h$  sa hauteur celle-ci étant égale au plissement de la peau au niveau du nodule diminue du plissement normal, le tout divisé par 2. On peut constater que le volume de la lésion obtenue est une fonction linéaire du logarithme de la concentration de matière virulente.

L'antigène que nous utilisons avec Durand ne comprenait que des rickettsies purifiées par des centrifugations fractionnées.

Très rapidement nous avons adjoint les antigènes solubles constitués par les extraits de rickettsies et les extraits des tissus dans les quelles les rickettsies ont cultivé. Ces différents produits ont un pouvoir antigène au moins égal à celui des rickettsies. Nous avons aussi montré la baisse de ce pouvoir antigénique par chauffage. De plus nous avons vu que ces tissus, même après lavage finement broyés sont encore antigènes. Le vaccin comportait ainsi non seulement les

l'homme

*Essai de purification* — Mais nous voulions aussi pouvoir purifier rapidement nos suspensions et ainsi avoir un vaccin débarrassé de tout produit cellulaire. Pour le réaliser, nous avons essayé avec Mme Giroud et Mr Meunier un procédé basé sur la flotation et destiné à

aqueuse ainsi que les bactéries non acido résistantes. Les tissu les bactéries acido résistantes passent dans la phase non miscible. On obtient ainsi facilement des suspensions pures.

Nous avons décrit ce phénomène en 1943 quand nous nous sommes aperçus que la valeur d'un tel antigène purifié est inférieure à celle — — — — — C'est du reste une technique r les rickettsies

des différents antigènes que nous avons employés a u abord été donnée non l immunisation active des animaux cette preuve n'est pas très

tagnes Rocheuses virus que nous devons n t t t t t t t d'une grande utilité. En effet la souche que nous utilisons à Paris et que nous avons simplement conservé à  $-25^{\circ}$  sous forme d'organes

Mais Zinsser comprenait que, 'dans une alternative comme la notre', nous préférons une technique simple permettant, si cela était nécessaire, un grand rendement. La souris pour nous se révélait insuffisante, aussi avons nous cherché à utiliser de façon pratique d'autres animaux. Nous avons employé successivement le lapin puis, quand celui-ci nous a fait défaut, le chien adulte. Le poumon de lapin ou de chien dose dans la peau donnent des résultats équivalents.

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Même actuellement, comme nous avons pu le voir avec notre collaborateur Vargues, les poumons de contrôle dosés dans la peau montrent une réaction positive au 0,01 mg. Un centième de milligramme

que nous n'avons que très peu employée avec notre collègue Jude  
Le serum à des dilutions variables est mis en contact avec des sus

Après une infection bénigne de laboratoire le taux observé est en général de 1 200 et permet de l'authentifier même avec 1 ou 2 jours seulement de température, ce taux est pathognomonique lorsqu'on le constate 3 mois après une vaccination. Après une infection typhique évoluant chez un non vacciné, le taux d'agglutination varie entre le 1 1 000 et le 1 10,000 e. A ce point de vue nous insistons sur les phases non spécifiques que nous avons décrites avec notre collègue Jadin du Congo Belge. A la phase des anticorps maxima, les deux antigènes épidémiques et murins peuvent être agglutinés au même taux. Mais on peut différencier facilement ces sérums avant ou après cette phase non spécifique. Une vaccination préalable n'est pas nécessaire pour faire de telles constatations. Nous pensons qu'on peut interpréter cette phase non spécifique comme due à des lyses de rickettsies probablement partielles ou l'antigène seul périphérique est extrait.

Pour l'étude expérimentale de l'antigène nous utilisons le lapin, généralement celui ci répond aux trux de 1 640 au 1 1 000 avec 20 cc d'antigène.

*Reaction d'hypersensibilité* — Une des réactions qui nous a donné aussi des résultats intéressants est la réaction d'hypersensibilité à l'antigène épidémique.

E  
don  
hyp  
dans la peau 1/10 de cc d'antigène épidémique provoque à partir  
du derme soit  
sujets normaux la réaction ne dépasse pas 4 ou 5 mm tandis qu'elle atteint 40 ou 50 mm chez les hypersensibles.

Comme elle est surtout très précoce après les infections très bénignes ou même inapparaissantes des vaccinés elle a pour juger une immunité une grande importance. Elle met en évidence la prémunition de ces sujets sur laquelle a tant insisté Ed Sargent. Ce que nous pouvons dire c'est que tous les sujets présentant une nette réaction d'hypersensibilité sont immuns, comme nous avons pu nous assurer expérimentalement.

d'animaux, tuait de façon régulière le cobaye et le singe. Et nous avons pu voir que ces antigènes pulmonaires formolés faits au dépens de poumons de souris ou de lapins donnent une bonne immunité contre la maladie mortelle du cobaye et du singe. Les meilleurs résultats ont été obtenus par la voie intra dermique. Il n'était donc pas douteux que ces antigènes formolés se nait en fait en fait maladie mortelle.

Il y a une courbe thermique au-dessus de 39°8 et mesures au planimètre pour un type de vaccin nous avions 0,81, pour un autre 0,29 et la courbe thermique témoin donnait 1,17.

*Pouvoir neutralisant* — Tous nos dosages du début ont été faits avec notre test cutané de séro-protection nous n'insisterons pas les résultats.

pratique comparative de notre test cutané de séro-protection de la séro-protection sur souris par voie veineuse, péritonéale ou trachéale, nous a montré que ce premier test était vraiment plus simple, ne nécessitant pas le recours à des données statistiques. Ses résultats étaient constants à condition toutefois de se tenir à notre technique habituelle dose variable de virus connu en poids serum pur ou dilué.

Le sérum d'un vaccin neutralise en général 0,1 mg et 1 mg de poumon très virulent.

Mais la présence d'anticorps neutralisants dans un serum n'est pas un fait suffisant pour prouver d'une façon absolue l'immunisation. Il fallait l'épreuve expérimentale sur l'homme.

*Expérience spontanée chez l'homme* — Nous sommes à même de donner des résultats de vaccination avec épreuve et contrôle. Les sujets vaccinés l'étaient avec l'antigène formolé. Les contrôles furent fortuits. Deux personnes de passage au laboratoire se contaminèrent avec du virus épidémique conserve par voie pulmonaire sur lapin, nous ne manipulions à ce moment que ce virus ces contaminations furent probablement réalisées par voie respiratoire ou oculaire et ceux-ci firent des typhus graves.

Si l'on veut chiffrer leur maladie, on le peut dans leur cas en prenant l'intégrale de la courbe thermique au-dessus de 37°8 et mesurée au planimètre.

Dans ces conditions ces typhiques témoins ont des indices respectifs de 15,33 17,45 tandis que nos vaccinés contaminés manipulant des quantités considérables de produit virulent ont fait suivant leur sensibilité propre des maladies de type grippe bilatérale et allant jusqu'au simple accès fébrile et donnant des indices de 8,5 7,4 6,5 4,7 3,4 2,4 2,0 2,1 1,3 0,7 0,4 et dans une succursale de fabrication 5,2 2,0 1,0 0,7 tandis que 73% restaient sans aucune réaction.

*Agglutination des rickettsies* — On authentifiant aussi ces infections par l'agglutination des rickettsies faites suivant une technique un peu



Les antigènes extraits à l'alcool donnent de bons résultats, ils sont au moins équivalents si non supérieurs à ceux fixés par le formol.

ques à basse température et avec certaines concentrations.

Ne s'agit-il pas de la même chose que le formol n

Et nous concluons que si toutes les immunisations actuelles ou futures ne sont pas absolues, elles ont du moins complètement transformé la lutte contre le typhus des vaccinations même imparfaites ajoutées aux méthodes antiparasitaires chimiques que vous nous avez apprises, ont fait du typhus épidémique une affection historique

#### ABSTRACT OF DISCUSSION

Dr JONNY C. SYDNEY (United States), commentator. It is a pleasure to comment on Dr Giroud's paper on vaccination against epidemic typhus. The members of the Congress are doubtless familiar with the remarkable advances in our knowledge of effective vaccines which have been made in the past few years. I shall remark only on the developments in regard to vaccines containing killed *Rickettsiae prowazekii*. Although Drs da Rocha Lima and Weigl in 1918-20 demonstrated that infected human lice could be used in preparation of killed rickettsial vaccines, more feasible methods were not developed.

R. A.  
L. Tatera  
critonell

nt of work on rickettsial vaccines employed by the United World War was made from yolk. That Dr James Craigie and Topping and his associates developed a very successful technique for the potency testing of such vaccines.

This question of the potency of a typhus vaccine seems to me to be the crux of the matter, and I think it fair to assert that killed rickettsial vaccines of the epidemic type, whether derived from lungs as

et en dehors de toute autre possibilité de contagion et chez des sujets

*Resultats pratiques*—Les vaccinations qui ont été effectuées dans tous les camps de prisonniers français ont donné des résultats remarquables mais les contrôles épidémiologiques, qui sont toujours difficiles, n'ont pu être réalisés dans les conditions si spéciales où nous avons été placés. Nous avons bien eu de très nombreux comptes rendus des médecins français des stalags mais les résultats donnés ne peuvent pas être utilisés statistiquement. De toute façon, il n'y a pas eu mortalité par typhus chez les vaccins, ceux-ci ont fait des typhus bénins ou des formes frustes lorsqu'ils se sont infectés.

6 mois pendant toute cette période il n'y a pas eu de cas de typhus dans son stalag. Refoulés vers la Pologne, dans les camps de rassemblement successifs, il n'y eut pas de typhus constaté parmi les  
les  
au

ne  
ne donnait pas un résultat absolu, elle permettait de subir, sans grand

l'acide phenique

Des agents arrêtant la croissance des germes comme les sulfamides ou les thiazomides ne peuvent pas être utilisés pour la fabrication des antigènes.

Le sunnoxol à 2°/00, la gonacrine à 3°/00 conservent le pouvoir antigène, les agglutinines sont de 1/320 à 1/640 chez l'homme, le test de séro-protection est positif, tandis que l'oxylrésorcine (1%) ne vaccine pas. L'hexaméthylène tétramme à 10% donne quelques résultats. Par contre l'ether de l'acide p-oxycarboxybenzoïque à 5°/00 (Nipagine) provoque une bonne immunisation. Les cobayes vaccinés par une seule injection donnent des indices de 0,015 et les témoins de 2,5.

Avec G. Ciaccio, nous avons en outre utilisé ces temps derniers des extractions alcooliques et des précipitations.

Our laboratory experience was perhaps a better guide to the value of the vaccine. Previous to the introduction of vaccine we had 12 cases of typhus—most severe and one fatal. Since the introduction there have been six cases all mild—so mild indeed that the clinician

hesitates to give us a vaccine which will have the same protective value as say, yellow fever vaccine has against yellow fever. Does he regard it as a matter of increasing the amount of rickettsial antigen?

Dr M. RUIZ CASTAÑEDA (Mexico). I wish to congratulate Dr Giroud for his success in producing an excellent vaccine against typhus. I think that a good vaccine must contain as many rickettsiae as man can stand, which is estimated to be that amount that begins to produce unwelcome reactions. A better immunization is afforded by repeated doses of vaccine. This is in accord with what Dr Gear said. Therefore we must encourage the production of vaccine of high rickettsial content. The use of large doses of antigen has shown that even murine vaccines can protect against classic typhus if given in sufficient amounts and doses, a fact which has been corroborated by South African workers and by personal experience during a number of years. In this respect, Dr Veintemillas, of Bolivia, made interesting experiments which have been underestimated or have not been very well known. He protected humans against classic typhus by giving them three or four doses of murine vaccine and then infecting them experimentally, in the laboratory, with a good dose of classic typhus. He proved that these men were properly protected against the infection. Based on this experiment, we have produced bivalent vaccines in which we add a good proportion of murine rickettsiae. The egg method, of course, is excellent for supplying enormous amounts of vaccine. The 'lung' methods are also very good for those who prefer this type of vaccine.

Now, to give you an idea of how many rickettsiae can be obtained from the lung from a single mouse, I would like to recall one experiment of Mosser, as he described it during a recent lecture in Mexico

shows how many rickettsiae can be obtained from a single mouse lung

Dr PAUL GUOTD (France). Je crois comme je pense le Prof Felix que la question des antigens a la plus grande importance. Aussi ai-je, avec le Dr Ciacio, fait des extraits avec l'alcool, ethylique et methylique, à différentes concentrations et des précipitations à basse température avec l'alcool methylique. La chloromycine modifie aujourd'hui complètement le problème de la vaccination. Me permet d'envisager de nouveau une vaccination avec virus vivant.

in the methods of Dr Giroud and Dr Castañeda, or from gerbils, as in the method of Dr Gear, or from lice, by Weigl's technique, or

similar to those of Craigie and of Topping

It is satisfactory indeed to consider the fine results which have followed the use of killed rickettsial vaccines under actual epidemic conditions. Dr Giroud has mentioned the excellent results with his vaccine. The experience of the United States armed forces was entirely similar—no mortality and a striking reduction in the severity of the illness.

Dr J H S GEAR (Union of South Africa), commentator. I regard it as a privilege to comment on Dr Giroud's paper, and I was very much interested in it. It recalled to me the evolution of the vaccine which was produced at the South African Institute for Medical Research during the war. There we had only one vaccine, and it was

#### discussion

Possibly of some interest to the audience was our gerbil culture

some importance to the development of our vaccine was that these animals are the principal animal reservoir of plague in South Africa. An organization existed devoted to their extermination. We interfered in one stage in this process. Instead of being poisoned, they were caught by a simple trap. One hundred traps would catch 50-70 animals per night. A special squad caught up to 3,000 animals per week, which were used for the production of vaccine. The method was based on the Zinsser Castañeda method of preparation from white rats, the main difference in the result being that, as well as prolific growths of *R. mooseri*, prolific growths were obtained of *R. prowazeki*, the epidemic strains.

It was important to know whether South African strains of epidemic typhus were immunologically similar to European strains. A series of cross immunity tests and of serological tests revealed no difference between them. Accordingly, we had no hesitation in vaccinating the South African army operating in the Middle East with

amongst them there were 12 cases of typhus, none were fatal. Amongst the rest of the population, at the same time, there were over 1,000 cases, but the two groups were not exactly comparable.

for Respiratory Diseases, who received the specimen for study, was able to establish the disease quickly in guinea pigs, isolate the virus in chick embryos, and prove, by further immunological studies, that the agent was a rickettsia similar to that of Q fever.

Researches made in Italy among Allied troops, after my work in Greece became known, proved that the outbreaks of a disease in Italy diagnosed as atypical pneumonia were really outbreaks of Q fever.

Thus was Q fever for the first time recognized as a respiratory disease. Its geographical distribution today must be considered very large. The disease was recently described in Rumania (Combesco, 1947), and its presence may be considered as very probable in Asia Minor, according to our own recent researches.

#### RECENT EPIDEMIOLOGICAL INVESTIGATIONS

and spring excluded the possibility of tick transmission. Besides, its interruption during the hot season shows clearly that the inter-human transmission by sputum is not the exclusive mode. The evolution of the epidemic and its interruption, both occurring always at

To find out this source, we proceeded, beginning in the winter of 1946, to new epidemiological investigations, and this time we had the advantage of employing not only the experimental method but also the complement fixation test on serums.

Dr. Robert Huebner, of the National Institute of Health of the United States of America, was kind enough to perform this test on  
as peculiar  
the Balkan  
countries, and Asia Minor, where they are employed as domestic milk

ats in Greece and Asia Minor  
a positive test, but in a low  
titer. Later on, specimens of sera of goats from Athens and from various other districts of Greece and Asia Minor also yielded significant titers. But this test was shown to be useless for the diagnosis of the infection of goats and sheep, as it was found negative, with only one exception, on sera of goats and sheep which had exhibited a severe experimental or natural infection.

# Q FEVER, A RESPIRATORY HUMAN EPIDEMIC DISEASE IN THE MEDITERRANEAN AREA, DETERMINED A MILK-BORNE INFECTION FROM GOATS AND SHEEP<sup>1</sup>

J CAMINOPETROS, *Chief of the Experimental Medicine Service, Pasteur Institute, Athens, Greece*

The Q fever originally observed in the region of Queensland, Australia, has been up to today known in Australia and in North America as a sporadic human disease, localized in agricultural areas because of its transmission by ticks

The Germans recognized early the nosological entity of this epidemic bronchopneumonia. Its peculiar clinical and laboratory characteristics permitted its differential diagnosis from the common bronchopneumonias. That is why German physicians called it "Bal-

manifested by the death of the animal as well as by the creation of apparent lesions in the lungs and the pericardium of the guinea pigs. In the smears of spleen numerous organisms in compact masses were observed.

We maintained this strain for 15 months and through the courtesy of Dr Zografonitis, member of the Typhus Commission in Athens,

<sup>1</sup>In the absence of the author this communication was read for him by Dr Norman H Tepping.

### SUSCEPTIBILITY OF GOATS AND SHEEP TO THE VIRUS OF Q FEVER

In these experiments, animals from various districts of Athens and suburbs and a small number recently imported from Asia Minor were employed

*Experiment 1 (22 April 1947)*—One kid and one lamb were inoculated into the lung with infected human blood, one kid with sputum in the same way, and one kid and one lamb by nasal instillation of blood after narcosis with ether. Four young dogs and two young pigs were inoculated with blood into the lungs, and one young pig and two young dogs by nasal instillation of blood. For each series two guinea pigs served as controls. All kids and sheep exhibited, after an incubation period of 6–10 days, a high fever that lasted 7–12 days. Animals that had been inoculated into the lung, or had been infected by nasal instillation, presented symptoms of bronchopneumonia. The blood of infected lambs injected into guinea pigs proved to be infectious during the whole febrile period.

On 22 April 1947, 10 kids and 10 lambs were inoculated into the lung with infected human blood. On 23 April 1947, 10 kids and 10 lambs were inoculated into the lung with infected human blood.

subcutaneously with infected blood of guinea pigs, while the other two were inoculated subcutaneously with blood of the two kids of the first experiment. All kids presented, after 8–10 days, a severe infection. The fever rose frequently to  $42^{\circ}\text{C}$  and was accompanied by a strong shivering (chills).

At the place of injection there occurred an extensive inflammation. This inflammation subsided with the fever, which always fell by lysis. In smears from skin tissues of local inflammation numerous rickettsias were found.

Heart puncture, made on two kids for taking blood, revealed pericarditis. The blood

autopsy the  
with marked adhesions,  
In spleen smears were  
rickettsias

Simultaneously with the inoculation of these kids, we proceeded to the subcutaneous inoculation of two milk giving goats. Both developed a severe infection, which evolved exactly in the same way as with the kids, and their blood also was shown infectious to guinea pigs.

*Experiment 3 (9 June 1947)*—Four lambs were inoculated subcutaneously with blood from two kids of the preceding experiment. Two lambs were inoculated via the subcutaneous route and two lambs were inoculated cutaneously and severely infected.

For this reason, in our subsequent investigation as to the relation of goats and sheep with the human disease, we employed exclusively the experimental method. By this method we have been able to prove (1) That sheep and goats are very susceptible to the virus of Q fever, (2) that they serve as a source of infection because of a peculiar characteristic of their disease, that is to say, that the virus appears in their milk, and remains long after the end of the disease, and (3) that the infection of goats and of sheep is transmitted, above all, through the respiratory tract.<sup>1</sup>

Among British troops in Athens, although diminished in number, numerous cases of the disease occurred during the winter and spring of the year 1946-47. Cases among Greeks were also numerous. At the British Military Hospital in Athens we studied 40 severe cases, and among Greek soldiers 6 at the Rimini Hospital (Dr Kalitzantzis and Dr Papanicolaou), 4 at the Air Forces Hospital (Dr Trivizas and Dr Corombilis), and 12 among civilians (Dr Tsangridis, etc). The virus was recovered from the blood of 24 cases and from the sputum of 12 cases by injection into guinea pigs.

In nearly all of these cases the complement fixation test was performed and the result was found to be positive.<sup>2</sup> The test was also

Thirteen cases of bronchopneumonia were observed among British troops in Salonica. The complement fixation tests performed on four specimens were positive.<sup>3</sup>

The outbreak among British troops began in January and ended in June (January, 5 cases, February, 7, March, 9, April, 13, and May, 6).

.....

is remarkable that sheep and goats were herded in nearby pastures in the Grottaglie Air Base, Italy, where American soldiers were affected by the disease (Epidemiological Studies, American Journal of Hygiene, July 1946, p. 89).

<sup>1</sup> Although the complement fixation test proved to be of no use in these investigations yet we continued to try it in any case in man or animal. The results will be published in

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and negative in a few days *after the fall of the fever*. In smears of the conjunctiva and the cornea were found numerous intercellular masses of rickettsia. The injection of urines<sup>2</sup> of these animals into guinea pigs, repeated in the course of the disease as well as after its end, was always negative.

The goat milk, however, proved to be infectious to the guinea pig very early in the course of the disease, and it remained so during the whole lactation period. This fact, in connection with the transmission of the infection to goat and sheep by nasal instillation is of great importance, as it explains the presence and establishment of the Q fever in the form of a respiratory disease.

#### PRESENCE AND MAINTENANCE OF THE VIRUS IN THE GOAT MILK AFTER EXPERIMENTAL INFECTION

*Experiment 5*—We had the opportunity to inject into guinea pigs the milk of a goat (G) of the fourth experiment, on the eighth day of fever (30 July 1947). The injected guinea pigs developed, after incubation for a few days, a typical infection.

This experiment was repeated with milk from the same goat (G) on 12 August 1947, 2 days after the end of the illness, and at the same time with milk from the goat (ED) in which 3 days previously the illness had started. In both cases the result was positive.

In a third experiment (19 August 1947), 9 days after the disease of the first goat and on the tenth day of fever in the second goat the result was also positive.

In a fourth experiment (29 August 1947) we injected the milk of three goats, (G), (E), and (D). The results for all three were positive.

In a fifth experiment (18 September 1947) and in a sixth (6 October 1947) performed with the milk of the three goats (ED, G, D), the results were again positive.

The above experiments show definitely that the virus is present in the milk from the first days of the disease until at least 3 months after the end of the disease.

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4

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d a broncho  
above goats

at the Pasteur Institute. Her milk was found infectious to the guinea pig toward the end of the disease and remained so till the end of the experimental work.

Specimens of infectious milk, after having been kept in the Frigidaire at least 3 months, remained infectious, on the contrary, after souring, the milk was no longer infectious.

<sup>2</sup> The introduction of a thermometer into the rectum provokes a reflex mict on the moment the thermometer is taken out.

after an incubation period of 6-10 days and on the site of the injection there developed an extensive inflammation. The horses and mules exhibited an intensive inflammation of the conjunctiva accom-

local inflam

local inflam

mation

The blood of the lambs was infectious for guinea pigs at the beginning and the end of the fever. The same occurred with the blood of the horses and the mules. On the contrary, dog and cat blood injected into guinea pigs did not produce any infection.

The complement fixation test of dogs and cats was found to be positive, while the results on sera of horses and mules were negative.

#### EXPERIMENTAL INFECTION OF GOATS AND SHEEP IN THE FORM OF BRONCHO PNEUMONIA BY NASAL INSTILLATION OF THE VIRUS

*Experiment 4*—For this experiment the following animals were

pigs whose complement fixation test before the inoculation was found positive). The two kids and the two lambs had already been inoculated in the first experiment.

All these animals were tested by a direct nasal instillation of the virus. Some of these were also inoculated into the conjunctiva of the upper eyelid.

With the exception of the two kids and the two lambs which were reinoculated and the male goat with positive complement fixation test, all animals after an incubation of 6-9 days showed a severe infection accompanied by cough and dyspnoea. In two goats coarse rales were heard and in radio rains dense consolidations of the lungs were seen.

The animals inoculated into the conjunctiva exhibited an intense conjunctivitis. The eye lesions, however, disappeared after 2 months without important sequels.

The blood of the ram, which developed a severe infection,\* served for a nasal instillation to the milk giving goat (ED). Simultaneously we injected blood of the goat (G) into another young milk giving goat (A) from Old Phaleron. The latter was found immune. But

more productive on the sheep. Injection of nasal secretions of the goat (E) into the guinea pig was positive at the height of the disease.

\*The fever remained high for 10 days. Marked weakness appeared later and after 2 months the animal presented a paraplegia of the posterior trunks in consequence of which it died after 6 months.

result of the tested milk of the parent goat. The same results occurred with the five kids of the experimentally infected goats G and D.

Nine kids and five lambs of naturally infected sheep and goats, of the said flock, were found with fever, and their blood was also infectious to guinea pigs. These observations point out that the infection of newborn lambs and kids is transmitted by milk, so milk is the source of infection to these animals.

It remains to examine the role that ticks play in the transmission of the infection to goats and sheep.

It is to be remarked that ticks are not found on domestic goats and sheep in the suburbs of Athens but only on those of rural districts.\*

### SUMMARY

The seasonal incidence of Q fever in Greece manifested as a respiratory disease, occurs during the period from December to July.

The presence of virus in human blood and sputum is shown by the experimental infection of guinea pigs.

The great susceptibility of goats and sheep is demonstrated by inoculation of the virus.

The experimental infection of goats and sheep, in the form of bronchopneumonia, may be accomplished by nasal installations of virus.

A natural infection, in the same form, occurs in goats and sheep.

The virus is present in the milk of goats and sheep, experimentally or naturally infected, during the whole milk period.

After pregnancy the virus reappears in the milk of infected animals and may be transmitted directly to new borns.

Milk appears to be the source of infection in men.

The interhuman infection by sputum cannot be the main mode of transmission, because of the interruption of the disease during the hot season.

The outbreaks of the human disease are concurrent with the milking period of goats and sheep.

The manifestation of the disease in the form of bronchopneumonia may be attributed to the susceptibility of the respiratory system of both man and animal.

### ACKNOWLEDGMENTS

We must acknowledge our indebtedness to Dr G. Vernon, Dr G. Smith, pathologist of the British Hospital in Athens, Dr E. Kendall, medical specialist, Dr Catto, and Dr Skaffly, radiologist, for the aid they offered us in our research work.

\* All these facts concerning the infection of goats and sheep and the role played by their milk were reported to Dr. Rella Dyer, Director of the National Institute of Health, in a letter of 31 January 1948.

## RECOVERY OF VIRUS FROM MILK OF GOATS (MALTA RACE) BRED IN SMALL FLOCKS IN THE AREA OF ATHENS

four goats

On 28 August 1947 we took specimens of milk from eight goats of another flock (Eden, Old Phaleron) and with these, mixed two by two, four guinea pigs were inoculated. One of these guinea pigs fell ill after an incubation of 9 days

On 14 January 1948 we tested again the infectivity of milk of goats  
 six goats  
 1 carriers  
 week be

On 2 February 1948 we examined the milk of two other sheep of the same flock, which had also presented a severe bronchopneumonia, and in both the virus was recovered from their milk.

All these sheep had recently been imported from the island of Chio On  
and  
also

From the spleen and liver of these two embryos was injected into guinea

tive results

### MAINTENANCE OF THE VIRUS IN THE MILK AFTER PREGNANCY AND INFECTION OF NEW BORN

fever, transmitted a typical infection to guinea pigs. An indirect proof of the nature of this kid's infection was obtained by the positive

## SCRUB TYPHUS

JOSEPH F. SADUSK, Jr., M. D., *Associate Medical Director, Prudential Insurance Company of America, Newark, N. J.; formerly Executive Officer, United States of America Typhus Commission*

During World War II, the Allied Forces were confronted with the problem of scrub typhus, or tsutsugamushi disease, a disease hitherto unknown to our medical officers except as a textbook curiosity. At the beginning of the war, it was not generally realized that the disease was widely distributed through the Asiatic Pacific area. Present knowledge indicates that scrub typhus exists throughout the region ranging from northwest Honshu (Japan) and the Pescadores, down along Indo China, through Burma, India, Ceylon, the Maldives, and the Federated Malay States, into the Dutch East Indies, New Guinea, the Bismarck Archipelago, the Solomons, and even into many of the Philippine Islands.

Failure to realize the wide distribution of this disease prior to the

East Indies, "scrub typhus" and "bush typhus" in New Guinea, and "coastal fever" and "Mossman fever" in North Queensland, Australia. The investigations of Schüffner, Walch, Keukenschrijner, and Kouwenaar in the Dutch East Indies, Fletcher, Lewthwaite, and Sivoor in Malaya, and Gunther in New Guinea during the first three decades of this century leave little doubt that the clinical pictures described under these terms were scrub typhus.

You will recall that scrub typhus, probably more correctly known as tsutsugamushi disease, is a specific rickettsial infection due to the *Rickettsia orientalis* (syn *tsutsugamushi*). It is transmitted to man by the larval forms of certain mites, or chiggers. The disease clinically resembles the other rickettsial diseases except for the presence of an ulcerative and necrotic lesion called an eschar. This primary lesion appears at the site of the attachment of the mite vector and is similar to the *tache noir* of *fièvre boutonneuse*. Agglutinins for the Kingsbury (ONK) strain of the proteus bacillus appear in the patient's serum during convalescence.

During World War II scrub typhus appears to have been first encountered by the Australian and United States Army Forces operating in the vicinity of Port Moresby and Milne Bay, New Guinea, in October 1942. As the zone of combat moved over the Owen Stanley Range to the north coastal plain of Papua in the vicinity of Buna and

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that a minimum of 636 fatalities (table 2) have been reported, and over all case fatality rate of slightly less than 6 percent. Studies show, however, that case fatality rates have ranged from 1 percent to as high as 28 percent in single epidemics.

TABLE 2.—Deaths from scrub typhus during World War II<sup>1</sup>

Theater and force	1942	1943	1944	1945	Total
New Guinea and islands					
U. S. Army	0	48	165	19	232
U. S. Navy		3	20	3	26
Australian forces	40	112	3	27	182
South and Southwest Pacific					
U. S. Army	0	0	0	0	0
U. S. Navy		0	0	0	0
Australian forces	1	8	2	0	11
Philippines					
U. S. Army			0	10	10
U. S. Navy			1	1	2
Southeast Asia command					
U. S. Army		0	18	39	57
British		1	25	13	39
Chinese Army					
Total	41	172	210	105	528

<sup>1</sup> Data from Dr. C. B. Philip.

and also New Guinea

this organism was also isolated from eight *R. flavipes* and from four tree shrews, *Tupaia belangeri*. It is of interest that the last animal is an insectivore and not a rodent. Thus, a Philip points out, the zoological range of naturally infected mite host is definitely broadened.

Our Typhus Commission studies in New Guinea failed to confirm the hypotheses by Heald of the susceptibility and natural

Gona during November and December, the number of cases began

Solomons, in Burma, India, the Maldives, Ceylon, and even in the Philippines, where the disease had not been hitherto described

As a result, the efforts of the Australian, British, and American investigators were marshalled to meet the threat. Virologists, epidemiologists, clinicians and entomologists pooled their efforts to seek a solution for the prevention and treatment of this disease. So great

to all those investigators engaged in studies on scrub typhus during this period

*Vital statistics during World War II*—First, the collection of such statistics as are available indicates that a minimum of 10 331 cases (table 1) of scrub typhus were encountered among the Allied Forces during the war period 1942-45. Of this number, 3,184 were in the

TABLE 1—Cases of scrub typhus during World War II<sup>1</sup>

Theater and force	1942	1943	1944	1945	Total
New Guinea and islands					
U. S. Army	33	941	4,357	80	5,411
U. S. Navy	0	47	360	198	605
Australian forces	186	1,870	602	181	2,839
South and Southwest Pacific					
U. S. Army	1	0	26	2	29
U. S. Navy	9	1	3	0	13
Australian forces	3	180	132	31	316
Philippines					
U. S. Army			13	271	284
U. S. Navy			35	63	98
Southeast Asia command					
U. S. Army		56	610	301	967
British Army	7	637	3,801	1,072	5,497
Chinese Army		17	202	130	349
Total	222	3,629	10,162	2,318	16,331

<sup>1</sup> Data from Dr. C. B. Philip.

<sup>2</sup> Includes Assam but excludes remainder of India, data for which are not available for comparative purposes. Records show 1942-45 totals as follows: India, 420 cases; Ceylon, 799 cases; Maldives and Diego Garcia, 720 cases (plus 79 cases in British Navy).



likewise appeared to offer protection in animals. Particular mention should be made of the egg yolk sac vaccine of Cox, the tissue-culture vaccine of Plotz, and the lung spleen vaccine of Smadel prepared from intravenously infected white rats and mice.

*Chemotherapy*—Shortly before the end of the war, Tierney demonstrated the remarkable effectiveness of para aminobenzoic acid upon the clinical course of scrub typhus in the human. His studies were undertaken as the result of basic laboratory investigations which

revealed that the drug survived in high percentage despite heavy infection with *R. mooseri*. This important discovery was quickly confirmed by others. Additional investigations showed that this drug exhibited considerable antirickettsial activity not only against epidemic typhus, murine typhus, and Rocky Mountain spotted fever, but also against scrub typhus under laboratory conditions.

It was found that this drug, when administered early in the disease in doses of from 24 to 36 grams per day, shortened the course of fever, ameliorated symptoms, and decreased the mortality rate.

This new agent may play an important role in the treatment of not only scrub typhus, but also the other rickettsial diseases.

*Repellents and area control*—Another striking advance in scrub typhus, from the practical standpoint of prevention of the disease, is the development of mite repellents, or miticides as some insist this group of chemicals be termed.

Early in the war, investigators in the Orlando laboratory of the Bureau of Entomology and Plant Quarantine of the Department of

Agriculture found that dimethyl phthalate was an extremely toxic repellent. Since the experience of the average person in the United States and the Orient that chiggers were only a nuisance in this hemisphere, little attention was paid to the discovery. However, the development of scrub typhus among our armed forces in New Guinea in rapidly increasing incidence during 1943 reopened interest in the subject. By this time, thanks to the early efforts of an Australian entomologist, Captain McCulloch, dimethyl phthalate and

fection in the Australian bandicoot. Indeed, this marsupial appears to possess a considerable degree of natural immunity to the infecting organism.

white rats, to isolate *R. orientalis* from the blood for 22 days and from the brain for 98 days after initial infection. The British scrub typhus team in Burma confirmed these findings with laboratory infected native rats. They found evidence of infection of the blood stream and brain in these rats for 74 and 99 days, respectively. Fox and Peterson, in the Rockefeller laboratories of the International Health Foundation, were able to isolate the causative organism from white mice, which had recovered either naturally or by treatment with chemotherapeutic agents, for periods almost up to a year. Thus the important fact that *R. orientalis* can live in symbiosis with its rodent host for prolonged periods appears to be well established.

*Etiological agent*—Considerable progress has also been achieved in basic studies upon the etiological agent, particularly in relation to its ability to evoke protective and complement fixing antibodies. Many strains were isolated from mites, animals, and man, in New Guinea.

broad antigenic groups. However, it should be noted that an animal

tion for general laboratory use but also the theoretical effectiveness of a vaccine. Although considerable effort was expended during the war years in the production of a prophylactic vaccine, the efforts were successful only in bringing this problem to the point of successful protection of laboratory animals. A vaccine prepared by Fulton and Joyner came too late for extended field trials, although such were

that it was put into the end of the war. Samples of vaccine were brought out in the United States on an experimental basis. They

## ABSTRACT OF DISCUSSION OF PAPERS BY CAMINOPETROS AND SADUSK

Dr JOAO FRAGA DE AZEVEDO (Portugal) Dernièrement le Prof Fernando Fonseca, le Dr Manuel Pinto et moi même nous avons trouvé à Lisbonne 18 cas de fièvre Q dont le diagnostique a été fait par l'inoculation au cobaye et par la réaction de la fixation du complément avec l'antigène de la maison Lederle préparé avec la *R burneti*. Par des études épidémiologiques nous pensons aussi que le lait de vache peut être un des véhicules de la *Rickettsia* parce que avec le sang d'un de ces animaux nous avons obtenue une réaction de fixation du complément positive. Aussi nous avons obtenue une réaction positive dans un des employés de l'abattoir. Les recherches sur le fièvre Q se poursuivent en Portugal mais cependant je dois vous dire que les cas ont été sporadiques et que quelques uns ont donné une symptomatologie pulmonaire, tandis que d'autres cas ont donné une symptomatologie méningée et d'autres une symptomatologie comme celle de la grippe. Parfois les malades avaient une eruption cutanée de papules. Tous les malades ont eu de la fièvre très élevée, mais tous ont guéri.

Dr NORMAN H. TORRINO (United States) It might be of some interest to mention to the audience the recent history of Q fever in the United States. An outbreak of Q fever occurred among slaughter house workers and stockyard workers in Amarillo Tex. 2 years ago and some 6 months later an outbreak occurred in Chicago. At present there is an outbreak of Q fever in Los Angeles, over 900 cases have been reported in Public Health Reports. Drs Huebner, Shepard, Parker, Jellison, and Beck published a paper which reported the isolation of rickettsia of Q fever from the milk of sheep and goats in the Los Angeles area. This paper was submitted by Dr Caminopetros in 1937.

also dibutyl phthalate were receiving field trials in New Guinea. At the request of the Commanding General of the Southwest Pacific Area, an investigative group was dispatched to New Guinea in the fall of 1943 by the United States of America Typhus Commission and the Army Epidemiological Board. It was immediately evident to this

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be even superior to the phthalates in quick killing time and in re

benzyl benzoate

Application of sulfur dust, DDT and fuel oils to the ground have been tried for area control of mites but in general the results are not satisfactory from the point of view of permanence. Recent unpublished reports from the Orlando laboratory indicate that hydroxy penta methyl flavin and benzene hexachloride applied either as a dust or spray at dosages as low as 4 to 6 pounds per acre may eliminate mites from an area for at least a month after treatment.

#### SUMMARY

If sent from 1 - 2 - 2

These gains made during the last war, constitute another example of the brilliant progress and accomplishments of military preventive medicine.

In 1941, the disease became epidemic in North Africa. There were also numerous cases of poliomyelitis among the troops in this area at that time, and the virus of poliomyelitis was isolated from the faeces. Observers noticed many apparent similarities between infective hepatitis and poliomyelitis and after numerous intensive field investigations concluded that the hepatitis virus was present in the faeces of infected individuals and transmission occurred by means of faecal contamination of hands, food, etc. Van Rooyen (4) was unable to obtain permission to prove this theory by inoculation of human volunteers. However, as a result of Anglo American cooperation material collected by English and American workers in the Middle East was transported to the United States of America for investigation there.

Meanwhile, the disease had also become epidemic in civilians and military personnel in Great Britain and West Africa, and similar types of study were organized and carried out. In England, where the disease had been endemic for many years, the past and recent field investigations suggested that transmission was more a question of droplet spread than of faecal contamination. As a result of this conception, first nasopharyngeal and oropharyngeal secretions were tested, then urine, and last of all faeces, for the presence of an infective agent. In West Africa, as in North Africa, faecal contamination appeared to be the most likely mode of spread.

Despite the wide divergence both of origin of the stools and of the countries where they were tested, positive results of oral administration of such infected material to human volunteers was reported more or less simultaneously, in England (5) in rheumatoid arthritics aged about 30 to 40, fed stools from cases of infective hepatitis in England in the United States (6) in healthy young males about 20 to 25, fed stools from patients in the Middle East, and in West Africa (7) in natives fed stools from local cases. The urine from patients in

have been present in the urine tested in West Africa.

Several attempts to infect adults by intranasal and oral administration of nasal washings, gargles, and extracts of tonsillar and pharyngeal swabs of patients in the acute stage of the disease (from first day symptoms to first day jaundice) were unsuccessful, as was an experiment using material from school children with subicteric illness in a class where cases of jaundice occurred both before and after the cases of donor patients. Unfortunately, this work was terminated before material collected in the presymptomatic stage could be tested. It seems unfortunate that more attempts were not made to

## Session 3 INFECTIOUS HEPATITIS

*Tuesday, May 11—2 to 4 30 p m*

*Departmental Auditorium, Room B*

### RECENT ADVANCES IN INFECTIVE HEPATITIS AND SERUM HEPATITIS

**T O MacCALLUM**, *Virus Reference Laboratory, Central Public Health  
Laboratory, London, England*

#### INFECTIVE HEPATITIS (VIRUS A. HEPATITIS)

When the recent war commenced in 1939, it had already become apparent in certain quarters that the true catarrhal jaundice of Virchow was a very rare condition and that in the majority of instances the underlying pathology of clinical catarrhal jaundice was a necrosis of the parenchymal cells of the liver. This thesis was based on post

stages of the disease by Roholm and Iversen (1) in 1939 in Denmark, and later by workers in numerous other countries.

It has been generally accepted since about 1930 that the aetiological agent of infective hepatitis is a virus but this hypothetical agent has resisted all attempts to transfer it consistently to experimental animals. There have been isolated reports of transmission to various species from mice to primates and the developing chick embryo particularly in Germany, but none of these claims have been substantiated by other groups of workers. For this reason, as soon as it became obvious that infective hepatitis was becoming a serious wartime disease, as it was during previous wars, various workers turned to experiments in human volunteers in order to determine the possible mode of transmission and thus possible methods of control in the field and prophylactic treatment.

Voegt (2) in Germany made the first report of transmission of the disease to human volunteers by oral administration of duodenal juice, injection of blood, and possibly by oral administration of urine from jaundiced patients. At the same time, the disease became endemic among British troops in Palestine, and Cameron (3) transmitted the disease by parenteral injection of blood from jaundiced patients. These early findings confirmed the belief that the disease

under conditions. Certain pools of gamma globulin prepared from pools of adult blood in the United States appear to be effective as a prophylactic measure when injected intramuscularly into exposed individuals in doses of 0.15 to 0.3 ml per pound of body weight (14). Passive immunity may last for 6 to 8 weeks after inoculation.

### SERUM HEPATITIS (VIRUS B HEPATITIS)

In 1937, in England, jaundice was observed in a number of children several months after they had been inoculated with a batch of pooled measles convalescent serum which had been collected from apparently healthy individuals (15). Similar incidents were recorded at the same time in individuals inoculated with yellow fever vaccine containing apparently normal serum (16). We now recognize this condition, which has been observed following the parenteral administration of apparently normal human serum plasma or whole blood as homologous serum hepatitis (haemic) (17) or haematogenous hepatitis. There is considerable difference of opinion whether this disease, which cannot be differentiated either by clinical or histological examination from infectious hepatitis, is caused by the same virus as

experiments carried out in three different parts of the world. England  
the results  
hepatitis and  
I appeared

to be somewhere between 20 and 40 days in the former (confirmed by experiments in volunteers), while in the latter it was in the region of 60 to 150 days. However, when sera of certain patients presumed to be suffering from infectious hepatitis were inoculated parenterally into volunteers, the incubation period to jaundice was between 60 to 120 days. It is possible, as Aycock (18) and others suggested that this prolongation is due to the presence of a certain amount of antibody in the inoculum. In other instances serum from infective hepatitis has given the usual short incubation period when administered either by the oral or parenteral route, though the attack rate in the parenterally inoculated group was less. By contrast, pools of known icterogenic serum which presumably contained virus B and had an attack rate of approximately 50 percent by parenteral route, failed to produce disease when given orally or intranasally. Further, this high attack rate by parenteral inoculation was obtained in a com-

transmit the disease by administration of such material, for if there is an analogy with poliomyelitis, as suggested so frequently, one would expect to find virus in this site late in the incubation period before onset of symptoms, as was recently demonstrated in poliomyelitis.

There have been several small localized outbreaks of an explosive character, which appeared to be due to contaminated river or well water, reported from various countries (8, 9). There have also been

virus 6 weeks after the closure of the camp where this epidemic occurred. The presence of the virus was demonstrated by oral administration of the well water to volunteers.

Thus, up to the present time, faeces and possibly urine are the only proved sources of infection for natural transmission of the disease, and blood in exceptional circumstances, and the onus rests on those who consider the upper respiratory passages as a source of infection, to prove their case.

*Diagnosis*.—The lack of a susceptible experimental animal has prevented the development of any specific laboratory test for diagnosis, especially of the mild type of case which may be anicteric. Attempts to concentrate the virus in blood or stools, or in tissues of fatal cases, by physicochemical methods, have so far been unsuccessful, even for the preparation of a specific complement fixing antigen.

However, some of the following tests are even these, and thymol turbidity tests, may also be used to differentiate cases of obstructive jaundice from hepatitis. Though not without risk, the improved technique of liver biopsy may, in selected cases, be a useful adjunct when other tests fail in differential diagnosis of possible obstructive jaundice. In spite of all these experiments and with full knowledge of their results, little success was attained in preventing spread of the disease even in the late stages of the recent war.

*Control*.—It is now generally accepted that there are several sick but anicteric cases for every icteric one, particularly in an epidemic, and possibly there are even symptomless infections. A question of considerable importance is whether there are carriers, symptomless or otherwise. The evidence available (11) from a small number of human experiments suggests that patients do not excrete virus for more than a week or two after the onset of jaundice, but no experimental information is available regarding carriers.

resistant to  
The virus



scale to maintain survival of the agent by inoculation unless healthy carriers existed. The fact that in many instances where the donors could be traced they were known to have been well at the time they supplied the blood but in some instances had suffered from jaundice at some former time, usually several years previous to the present hypothesis. I

hepatitis with

onward in several donors who had been infectious in the acute stage have been unsuccessful. Also the attack rates in volunteer inoculees indicate that infection with virus B has been uncommon in England and in the United States in the past. Experiments with washings of upper respiratory passages also suggest that it may be possible to maintain the disease in nature, though undifferentiated clinically from epidemic or endemic infectious hepatitis. It is very important that we should try to learn the frequency of distribution of this agent among the population and what type of individual is liable to be harbouring it.

further advance that is made in our knowledge may be dependent upon epidemiological studies.

**Control.**—Control of this disease depends upon the development of some rapid tests for detecting the agent in samples of serum or a ready means of inactivation. The former is still beyond our grasp, but ultra violet light radiation may be the answer to the latter. Oliphant (25) and his colleagues produced suggestive results with varied types of apparatus. It certainly seems feasible that sera or plasma may be dealt with in this manner, which would provide a continuous flow of bacteriologically sterile, nonicterogenic sera in a thin layer. The agent has been found in sera after exposure to 56°C for 1 hour and 0.25 percent phenol for 14 months.

Prophylaxis of possible infection in the incubation period by injection of gamma globulin prepared from pooled adult serum has

one hospital, at 30 days and 60 days after treatment, the attack rate was slightly less than in untreated alternate cases and the incubation period appeared to be prolonged in those cases which occurred (26). But other trials have been unsuccessful. No attempt has been made at prevention by gamma globulin prepared from convalescent serum from known cases. Since this disease appears to be much less wide spread than infective hepatitis, antibodies to it must be relatively limited in distribution and one would not necessarily expect to find them in an ordinary pool of adult serum.

infectious hepatitis (19)

taining virus B. Two of seventeen recipients developed jaundice approximately 100 days after inoculation. Recipients received several doses of washings collected from donors at different periods of the preicteric stage. Results suggested that the suspected washings were collected nearly 4 weeks before the appearance of jaundice. These results are, of course, not conclusive, but give a lead, especially as

colleagues (21), the agent was recovered from the blood stream 34 days after intradermal inoculation and 60 days before jaundice.

One should point out here the apparent lack of hepatitis following the inoculation of gamma globulin from pooled plasma in the treat

homologous but not to the heterologous agent is strengthened by a small number of reinoculation experiments in human volunteers (23). Though the groups are small, the evidence is, on the whole, clear-cut. The one exception is the experiment by Oliphant (24) in which individuals convalescent from virus B infection were resistant to parenteral inoculation of serum from a patient suffering from a presumed virus A hepatitis. The possibility arises that this latter case was in fact an infection with virus B. A number of other observations and experiments suggest that infection with virus B even increases susceptibility to virus A but the converse has not been observed.

All these transmission experiments suggest that there are in fact two unrelated agents and that in the majority of instances virus B causes a mild or inapparent infection and is responsible for most incidents of serum (haematogenous) hepatitis. Previous to the recent war blood and blood products were not used on a sufficiently large

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*Treatment*—The fatality rate in this condition has tended on the whole to be higher than that of infectious hepatitis, perhaps this is due to the relatively subnormal state of the patient who is already suffering from wounds or debilitating illness when the icterogenic

in apparent extremis when it was administered

### SYRINGE TRANSMITTED HEPATITIS

Ever since the introduction of the salvarsan therapy of syphilis a small number of cases of jaundice have occurred at varying stages of the treatment with different arsenical preparations. Some of these

planned experiments in clinics (27, 28) and human volunteers (29) have now shown that the majority of these cases are caused by transmission of the hepatitis virus or viruses in blood left in syringes or needles imperfectly sterilized between patients and that they may occur in any clinic where multiple injections or venepunctures are being carried out.

In conclusion we can say that though they may have been slight, advances have been made in our knowledge of what we must do to reduce the infection with the virus or viruses of infectious hepatitis in the field. We have also learned that a certain risk is attached to transfusion with large pools of adult plasma (or serum) and for this reason such products should not be used indiscriminately.

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tinguishable, there are certain apparent differences between them. These differences are concerned with length of incubation period, location of virus in the body, period of infectivity, route of experimental transmission, and lack of cross immunity. Whether these differences indicate actually different diseases or variant forms of a single disease is as yet, undetermined (15-17).

The discussion of certain of these problems is contained in the presentations of Dr MacCallum and Dr Neeff. This paper describes the clinical and epidemiologic aspects of the naturally occurring disease, infectious hepatitis.

### CLINICAL COURSE OF DISEASE

Infectious hepatitis is primarily a mild disease of children during peacetime (18), in contrast with the high prevalence and greater severity among young adults during war (19, 20). Although complete proof of the identity of the diseases, which appear both sporadically and in epidemics in children and young adults, is not established there is no reason to believe that they differ (18). In most epidemics

from hepatitis induced by a strain of virus obtained from the stools of children with the disease, were immune when re inoculated with a strain of virus derived from the stool of a soldier who contracted infectious hepatitis in Sicily (17).

The disease may usually be divided into two phases: preicteric and icteric, although an undetermined number of patients have hepatitis without jaundice. Anorexia is the most common early symptom, and weakness, nausea, abdominal

aches and pains. Physical

conjunctivae, posterior cervical adenopathy, and upper abdominal tenderness. Leukopenia with relative lymphocytosis is characteristic in the preicteric period, and bilirubinuria occurs before clinical jaundice is evident.

The icteric phase begins when the jaundice is prolonged deep

## CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF VIRAL HEPATITIS<sup>1</sup>

W PAUL HAVENS, JR., M D *The Jefferson Medical College,  
Philadelphia, Pa.*

The identification of the terms "catarrhal jaundice," "infectious hepatitis," "hepatitis epidemica," and "acute yellow atrophy" of the

in patients with this disease at necropsy. The development of the technique of biopsy of the liver by Iversen and Roholm (7, 8) and its subsequent use by others (9-11) in recent years made it possible to demonstrate clearly that the essential lesions in infectious hepatitis are

general was slow to deny the early concept of Virchow, and only recently has the actual pathogenesis of the disease been widely appreciated.

During the years of World War II, the importance of infectious hepatitis as an epidemic disease initiated numerous studies. MacCallum and Findlay and their associates in England, and American investigators including Paul, Stokes, Francis, Neefe and Havens,

covered (12-14). The available information now suggests that there may be variant forms of viral hepatitis, and at least two forms of disease are known.

<sup>1</sup>This investigation was conducted in part with the aid of the Commission on Virus and Rickettsial Diseases, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

endemic proportion  
spring. Since it  
somewhat higher  
1946 and 1947.

and the disease has apparently been diffusely spread throughout the command. The exact explanation of this endemic pattern is unknown. The possibility exists that the artificial transmission of virus by improperly sterilized stylets, needles, or syringes employed in such cases or administering using such a regular

*Age*—Infectious hepatitis is primarily a disease of childhood in the civilian population, and the highest attack rates have been seen between 10 and 14 years. Under proper conditions, young adults up to 30 are very susceptible. Among American troops in the Mediterranean, under 30

infectious hepatitis of men under 30 years (20). It is not determined whether immunologic or constitutional factors conditioned this response. The question has been raised as to whether difference in exposure among younger and older troops, as determined by the fact that men under 30 were more likely to serve in frontline battle, might account for this. That is on

ing epidemics may occur, although the usual experience is the occurrence of straggling outbreaks spread out over a period of 2 to 4 months. Family and institutional outbreaks are common. In the former, it is usual for hepatitis to occur in one member of the family, followed in 2 to 4 weeks by subsequent cases.

periods of contact  
1 family outbreaks  
and that when troops entered an area where infectious hepatitis was endemic, as in the Mediterranean littoral, large outbreaks frequently made their appearance within the next 1 to 2 months. McFarlan (19) suggested that the straggling course of epidemics is characteristic of a mildly infectious disease which is spread by contact and has a relatively long and varied incubation period. Gauld (20) described

the  
pos  
the

### EPIDEMIOLOGY

a few places, notably the Scandinavian countries, so that much of the information available has come from descriptions of epidemics large

defining more accurately the natural history of the epidemic disease

*Geographic distribution*—Reports from such widely separated

record of high incidence (22). In particular, the Mediterranean littoral has had a prolonged and high endemicity with severe epidemics among foreign troops stationed there during World Wars I and II.

*Season*—The prevalence of epidemics in the autumn and early winter months, with a decline in incidence during the spring and summer, has been observed in many different parts of the world. Kligler et al (23) have suggested that this seasonal trend may result from the crowding and closer personal contact which frequently occur at this time of the year. Under proper conditions, however, epidemics may occur at any time, as demonstrated (23) by an outbreak among young immigrants to Palestine, reaching a peak in June.

Hepatitis may also occur throughout the year with little apparent



in view of the fact that this disease may be transmitted to man by the parenteral inoculation of as little as 0.01 cubic centimeters of infectious serum (22). The possibility of mechanical transfer of infectious material by flies has been advanced by Kirk (31) and Trussell (32) in their descriptions of outbreaks among New Zealand and American troops at El Alamein and in the South Pacific area.

Lastly, the possibility of artificial transmission of infectious hepatitis merits consideration. The presence of hepatitis virus in the blood of patients, its high degree of resistance to ordinary procedures of cleansing, and its infectivity by parenteral inoculation suggest the possibility that it may be transmitted accidentally more often than is recognized.

*Immunity*—Epidemiologic evidence is supported by a limited amount of experimental data which suggest that a degree of immunity does follow infection. In civilian life, Pickles (33) and Lasner (34) in England have called attention to the fact that long intervals occur between epidemics in villages. Experimentally, both Havens (16) and Neefe et al. (17) showed that volunteers convalescent from experimentally induced infectious hepatitis were immune when reinoculated with the homologous strain.

The natural history of the disease is in accord with the concept that an attack of infectious hepatitis may be a disease of the liver and is not a disease of the blood.

among susceptible immigrant populations when introduced into an area where the disease is endemic and where the native population has acquired immunity (19, 23). The mildness of the disease and the possibility that infection is far less likely to result in subsequent illness than in the case of infectious hepatitis (19, 23) and Stokes and Neefe (24) have suggested that the disease may be a disease of the liver and is not a disease of the blood.

immunity follows an attack and that infection may occur more frequently than is diagnosed. Gauld (38) reported the incidence of infectious hepatitis as 42 per 1,000 among seasoned American troops in the Mediterranean theatre (1944-45), compared with an incidence of 109 per 1,000 among reinforcements.

It is difficult to evaluate the clinical and epidemiologic data which suggest that the disease may occur in 3 to 5 percent of the population and that it may not be solid and that it may contain nonspecific or even latent virus. The importance of the host, may be of importance. Unfortunately, it is not yet possible to determine whether such second attacks represent actual reinfection with the same virus or infection with another strain of hepatitis virus.

British officers in the 1942 epidemics in the Middle East had attack rates 47 times as great as enlisted men (19) Gault (20) has re

tious hepatitis spreads are not known although there is epidemiologic and experimental evidence to indicate that some form of person to person contact is frequently operative It is not unlikely that more than one manner of spread are effective, and that epidemics result from different combinations of various factors

The fact that virus is in the feces and may be transmitted experimentally by feeding such infectious materials suggests that the intestinal oral route may be of considerable importance It is of particular interest in this regard that when epidemics of this disease

A number of presumably water borne (20-26) outbreaks as well as food borne (29) and milk borne (30) epidemics have been described although there is no evidence that these are the most common modes of transmission Of particular importance was the demonstration by

Neeff and Stokes (26) of hepatitis virus in water obtained from a well in a children's camp in Pennsylvania during an epidemic of the disease.

Attention has also been directed to the respiratory route as a possible way of spread of infectious hepatitis. The clinical observation of symptoms and signs of disease of the upper respiratory tract in a certain percentage of patients at the onset of infectious hepatitis, as well as the increased incidence of the disease during the fall and

conclusions.

The possibility of transmission by insects either by biting or by mechanical transfer of infectious materials, requires consideration

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# SUMMARY

As a result of investigations of recent war years, a better concept of the natural history of infectious hepatitis has been established. Certain aspects of the clinical course have been more clearly defined, and the actual pathogenesis of the disease is now more widely appreciated. Infectious hepatitis has been classified as a disease of viral etiology which may be spread by the intestinal oral route and prevented by passive immunization. Epidemiologic and experimental data suggest that two forms of viral hepatitis exist. The exact relationship between these two types of disease is not known, although the demonstration of certain differences between them suggests that, although they may be closely related, they are not identical.

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of the Surgeon General, United States Army, were fortunate in securing from different sources, early in the period of investigation, one strain of hepatitis virus that was representative of so called infectious (or epidemic) hepatitis and one that was representative of the classic form of homologous serum hepatitis. For convenience in reference, the strains associated with infectious (epidemic) hepatitis will be referred to by the group term "virus IH" and those with the classic form of homologous serum hepatitis as "virus SH" (5)

Some of the properties of our two strains of hepatitis virus, as demonstrated by inoculation experiments in human volunteers (4-6), are summarized in table 1. Similar experiments conducted simultaneously and independently by Havens, Paul, and their associates with two other hepatitis viruses obtained from different sources yielded the same general results (1-3)

TABLE 1—Observations on hepatitis viruses IH and SH as demonstrated by transmission experiments in human volunteers

Observations	Virus IH	Virus SH
Usual type of onset	Abrupt febrile sharp onset of jaundice before laboratory evidence	Insidious, afebrile laboratory signs may precede clinical signs 2-4 months
Protective effect of human immune serum (gamma) globulin against	(+) (+)	(-) (-)

this disease in contrast with the comparatively sharp onset of SH

evidences of hepatic disturbance, such as bilirubinuria, urobilinogenuria, bromsulfalein retention, and serum flocculation of cephalin cholesterol emulsions, frequently preceded clinical symptoms. In contrast, well defined symptoms and physical signs were observed in virus IH hepatitis two to seven or more days before laboratory evidences of hepatic disturbance were obtained. Although these differences were consistent in the experimentally induced diseases in

\* Results negative but studies inadequate to warrant conclusions see text

## STUDIES ON THE ETIOLOGY AND EPIDEMIOLOGY OF VIRAL HEPATITIS<sup>1</sup>

JOHN R. NEEFE, M. D., *Associate in Medicine, Medical School and Hospital of the University of Pennsylvania, National Research Council Senior Fellow in the Medical Sciences, Hospital of the University of Pennsylvania, Philadelphia, Pa.*

During recent years, two forms of hepatitis have constituted problems of such magnitude to the armed forces or public health of practically all nations that they have achieved a high ranking among the more important medical problems of the day. In this country, one form usually has been referred to as infectious or epidemic hepatitis and the other as homologous serum hepatitis or jaundice.

The known characteristics of the causative agents warrant their tentative consideration as viruses, and the available information indicates that at least two different types of hepatitis virus, the exact relationship of which remains to be determined, are concerned. The clinical and pathological manifestations of these forms of hepatitis are indistinguishable, and clinical laboratory procedures permitting their specific differentiation have not yet been developed. Evidence for the existence of at least two types of hepatitis virus has been obtained only by means of a series of experiments in human volunteers, a procedure which obviously is not adaptable to clinical usage. As specific etiological diagnosis thus is not possible at the present time, the group term 'viral hepatitis' appears to be as specific a term as is warranted with present methods of diagnosis. Since hepatopathy also may be associated with other diseases presumably of viral origin (i. e., yellow fever, infectious mononucleosis, etc.) but usually is not the primary feature of these diseases, the types under consideration possibly would be more satisfactorily referred to under the tentative group term "primary viral hepatitis."

### CHARACTERISTICS OF HEPATITIS VIRUSES

Extensive studies of the properties of certain hepatitis viruses under a variety of experimental conditions have been conducted by Havens, Paul, and their associates at Yale University School of Medicine (1-3) and by our group including Stokes, Reinhold, Gellis, Blanchard, and others at the University of Pennsylvania (4-6). Both of these groups, working independently under the auspices of the Army Epidemiological Board, Preventive Medicine Service Office

<sup>1</sup> From the Nutritional Service of the Department of Pediatrics and of the Gastrointestinal Section of the Medical Clinic, Medical School and Hospital of the University of Pennsylvania. These investigations were conducted under the Commission on Measles and Mumps and the Commission on Virus and Rickettsial Diseases, Army Epidemiological Board, Preventive Medicine Service Office of the Surgeon General, U. S. Army.

for the presence of hepatitis virus. Thus, it appears that the presence of virus IH in biological materials would best be demonstrated by oral administration whereas virus SH would best be detected by parenteral inoculation. These factors should be considered in the interpretation of human transmission experiments in which negative results were obtained with materials tested for the presence of virus by only one route of administration.

In our studies, virus IH was consistently demonstrated in blood and feces obtained from volunteers during the preicteric and icteric stages of virus IH hepatitis. Attempts to demonstrate this virus in nasopharyngeal secretions and urine obtained during these stages of the disease were unsuccessful (5,15).

Virus SH was shown to be present in the blood of volunteers inoculated parenterally with this virus during the active stage of the disease and also during the interval between inoculation and the recognized onset. Feces, nasopharyngeal secretions, and urine obtained from volunteers with virus SH hepatitis were tested for the presence of the virus, the oral route of inoculation being used with negative results. As plasma known to contain the virus failed to induce the overt disease when administered by the oral route, the negative results with the other materials tested by this route afford no definite evidence concerning the presence or absence of this virus.

Perhaps the most convincing evidence of a difference between these

to develop overt hepatitis appears to warrant the conclusion that resistance to reinfection with the homologous virus had resulted from the initial infection. Volunteers so demonstrated to be resistant to the homologous virus were not resistant to the heterologous virus (IH or SH). The demonstration in the human test tube of resistance to the homologous virus and lack of resistance to the heterologous

with either virus IH or SH would

effect of human immune serum (gamma) globulin against hepatitis due to these viruses. The ability of gamma globulin to prevent the virus IH type of hepatitis when

healthy young volunteers, the literature indicates that the type of onset has not been sufficiently uniform in various outbreaks of the two forms of hepatitis to permit its use as a means of distinguishing between them (10, 11)

A consistent difference in the interval between inoculation and the time of onset of overt hepatitis also was observed. Following inoculation with virus III, overt hepatitis developed within 15 to 37 days. It is emphasized that this interval range was the same regardless whether the route of entry (virus III in pooled serum) was oral or

virus presumably would be associated with a prolonged incubation period when injected parenterally but not when administered orally.

After parenteral inoculation with virus SH, the time of onset of overt hepatitis was consistently between 2 and 4½ months. This interval was not significantly influenced by considerable variation in the size of the inoculum. It is of considerable interest, however, that

(13) These episodes may be related to the demonstrated occurrence of viremia in this disease long before the recognizable onset of acute hepatitis. These transient indications of early activity probably would be recognized only if the infected persons were under constant

mental conditions, they have proved to be useful in indicating which volunteers subsequently would be likely to develop the overt disease.

(14) No such phenomena were recognized during the interval between inoculation with virus III and the onset of the overt disease.

Results following the introduction of viruses III and SH into volunteers by different routes are of interest and may explain some of the

route of entry was oral or parenteral. With our strain of virus III the incidence following oral inoculation was considerably higher than that following parenteral inoculation.



It was found that the male and female sections of the camp each had a deep driven well as a source of drinking water, but due to inadequate supply in the male section, water from the well in the girls' section had been pumped intermittently into the reservoir in the boys' section, starting 1 week after the opening of the camp season. Bacteriological examination of water obtained from the well in the female section of the camp showed the presence of *E. coli* and provided evidence of contamination. Approximately 150 feet from this well was the cesspool which received the sewage from the cottage of the office worker who developed hepatitis 3 days after the opening of camp. It also received the sewage from the girls' infirmary to which all the early cases were subsequently admitted. A careful sanitary investigation (19, 20) of this cesspool months later provided satisfactory evidence of a connection between this cesspool and the well.

On the basis of the epidemiological observations alone, water from the well in the girls' section of the camp appeared to be the only potential carrier of the infectious agent that could satisfactorily explain the outstanding characteristics of the epidemic. The transmission experiments showed that the agent was excreted in feces, and the bacteriologic studies provided evidence of fecal contamination of the well water. Finally, the transmission experiments provided experimental evidence that this water also contained a transmissible agent that produced in volunteers an illness associated with hepatic dysfunction which was followed by resistance to infection with the feces hepatitis virus. These data appear to warrant the following conclusions regarding the pathogenesis of the epidemic. The virus was brought into the camp by the office worker and was introduced into the water supply by way of a connection between the cesspool, which received the sewage from the cottage to which she was quartered and the

accounts satisfactorily for the dissemination of the virus to the rest of the group.

It would seem, therefore, on the basis of the evidence presented, that the mode of studies work further

discussion is omitted for the purpose of conservation of space.

tion, when injected simultaneously with the plasma containing virus SH but at a different site, or when mixed directly with the infected plasma before injection (14). The — — — — —

present, therefore, recognition of the existence of at least these two types of hepatitis virus, the exact relationship between which remains to be determined, appears to be warranted.

### EPIDEMIOLOGICAL STUDIES

During the summer of 1944, a remarkable epidemic of viral hepatitis occurred in a large, isolated Pennsylvania summer camp for boys and girls (15). As this appears to be the only recorded naturally occurring epidemic in which experimental evidence of the method of

worth while to review briefly some of the observations made in connection with this epidemic.

This camp had been closed and uninhabited since the termination of the previous camp season in September 1943. Three days after the opening of the camp on July 1, 1944, a young female office worker quartered in the female section of the camp developed acute hepatitis. The male and female sections of — — — — — physical boundaries but, except

Sew  
which

worker, the epidemic — — — — — hundred and seventy-two persons at the camp developed hepatitis within a period of 13 weeks, the onset in 314 of the 350 occurring

as persons who had not had personal contact with one another, and (5) the apparent ease with which the infectious agent was acquired at the camp in contrast to the apparent lack of ease with which it was acquired by those subsequently closely exposed to infected persons after their return home.

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# ABSTRACT OF DISCUSSION OF PAPERS BY MACCALLUM, HAVENS AND NEEFE

weight of evidence brought forward - But merely to take the opposite point of view for the purpose of discussion, I often wonder if the evidence is sufficient to justify us in assuming that point of view. There are a number of factors about hepatitis to which these speakers have

is completed

Also the immunity effects in this disease are very remarkable. The infective dose cannot be ascertained and, if as little as the prick of a needle can produce the disease, I think the range of infection is likely to be a very large one and may be a factor which might account for

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## immunity in infective hepatitis

With regard to transmission my own small part in this has been limited to field studies, and I would like to conclude by referring to

## SUMMARY

In this paper, the group term "viral hepatitis" is used for those forms of hepatitis caused by similar specific infectious agents that ordinarily affect the liver predominantly and produce the syndromes commonly designated as infectious (epidemic) hepatitis and homologous serum hepatitis. The available information justifies the tentative classification of these agents as viruses and indicates that at least two virus strains, which may be different strains of the same virus or two different viruses, are concerned.

Under experimental conditions, one of these strains, herein referred to as virus SH, caused overt hepatitis in human beings 2 to 5 months after entry and consistently induced the overt disease only when entry was by the parenteral route. The other strain, referred to herein as virus IH, caused overt hepatitis in human beings 2 to 6 weeks after either oral or parenteral entry. The established modes of transmission of the SH type hepatitis virus are all artificial and are the result of accidental or intentional parenteral introduction of blood or blood

products. The IH type of hepatitis virus seems to be chiefly responsible for the occurrence of infectious hepatitis. Experiments on an epidemic of virus IH transmitted by drinking water are summarized.

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Dr JACK G MAKARI (Lebanon) I come from Lebanon where infectious hepatitis is prevalent. Newcomers are very likely to get it. The mortality is very high. I wish, however, to refer to one point which I was mainly interested in and which was brought out by Dr Neefe with reference to the deaths of preicteric cases and the early

a result of the presence of viruses, might produce a positive cephalin flocculation test. In 1946 we had reported on this test being positive in individuals before the development of icterus, as noticed in a small epidemic. Consequently, we have already suggested the routine use of this test by private practitioners to help in the diagnosis of preicteric and nonicteric cases of infectious hepatitis. We have also suggested that this test be used in all transfusion units and on all prospective blood or plasma donors to exclude infectious hepatitis, as well as malaria.

Dr I. A. GALLOWAY (United Kingdom) There are just one or two questions I would like to ask the experts who are working on this subject now. I gather that similar types of hepatitis have been recorded

titis and serum hepatitis. I took a little part in the investigation of the cases following the yellow fever vaccination of the American Army in 1942. During the course of those investigations it was shown that serum from acute phase cases contained antigenic bodies precipitable by an antibody present in the serum of convalescent cases. Subsequently, it was shown that the serum of monkeys inoculated with liver from a monkey dead of yellow fever contained an antibody which reacted with an extract of normal monkey liver in the precipitin test. It occurred to us then that in the pathogenesis of disease the following stages may have taken place. There was an infection of the cells of the liver. This infection made the liver cells, as it were, antigenic, or some part of the liver cell antigenic, and that produced an antibody, and, as a result of that antibody, one got a further degenerative process. I wondered whether the early stages of the infection, which Dr Neefe noted, were not the stages of the

showing urticaria, presumably of an auto antibody and general reaction, was much higher in the serum group than in the infectious hepatitis group, and I wondered whether Dr Neefe or Dr Havens could enlighten us on this point.

one or two early observations which suggested to us that the disease was spread by stool. The first was that of a soldier who received a very severe injury to his spine, was bedridden and had two army nurses to attend him. We warned both of them to avoid droplet infection and they religiously observed instructions. The man was severely bedridden and had incontinence of feces. Approximately 3 weeks afterwards, both nurses developed infectious hepatitis although there were no other cases in their group of about 30.

The second instance was that of 2 soldiers who were working in an ordnance depot at the height of the summer, when no infectious hepatitis was present among a garrison consisting of about 300. Both men went down with infectious hepatitis and inquiries revealed that the men had been engaged in sorting the clothes of killed New Zealand soldiers. The troops in the New Zealand regiment had sustained a severe epidemic of hepatitis.

There are some points which come to mind, and I hope that they will stimulate discussion.

Dr J. C. LORRUP (New Zealand). I work in western Samoa. Our experience down there with jaundice has been with a limited but definite epidemic extending about 12 years. Up to 1935 there were one or two cases of clinical jaundice reported each year. In 1935 something like 120 cases were reported, and about that rate has continued since then until 1947 when the incidence began to drop off. So far this year only one or two cases have been reported.

Now the interesting thing about the epidemic there was that among full Samoans the case mortality was about 40 percent, and among people of mixed blood and Europeans, while the incidence of the disease was about the same as that for the general population, the case mortality was low. The only deaths that occurred were in full Samoans and about 75 to 80 percent of those reported with clinical jaundice died. There are a number of complicating factors, and we thought of the possibility of infectious hepatitis in people whose livers were already damaged and there are a number of local factors which do cause liver

s

cases of all grades from simple infectious hepatitis with very few symptoms to the devastating crisis that comes on early and brings death from the acute necrosis before there is any sign of jaundice.

There are other factors that have worried us also, and the main one of those is the low protein diet of the people, which is being investigated now.

We are also aware that leptospirosis is prevalent in the rats in Samoa

we have shown positive anti-  
ferred with the study  
we are trying to make

up for that now

lieved") Believed to be one I think that is different I don't think there is any proof of it whatsoever It has never been confirmed, and the other Danish workers don't give much support to it We went into it, and I know many of us have tried to infect pigs What we found was that the liver of pigs on a low protein diet shows a picture exactly like the pictures that Andersen described for his infected pigs Our animals were on such a low diet that we even got a death in the controls, and yet we got no sign of any abnormality in the pigs which we had tried to infect with all manner of material

There are one or two points that I might make just here about the epidemiology My colleague, Dr McFarlan, who was unable to come to this meeting, has carried on a very intensive survey since 1944, when infectious hepatitis was made notifiable in nine counties of eastern England Accuracy, of course, is influenced by the mild cases not reported to the doctor The results have been fairly satisfactory The population of the region is about two and a half million, and the attack rate in 1944 was 1.3 per 1,000, and in the following years was 0.6, 0.7, 0.4 We got into this community at the end of the most severe hepatitis, and the inquiries showed that unfortunately the most severe epidemics had finished But the exact number of cases of

of these villages  
there was only a single case in the whole year Yet when the disease

1  
of these villages  
us stage  
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robably  
at least two The clinical picture is the same in both Dr Kaufmann, the histological picture in the liver happens to be the same, but the studies of cross immunity have been so consistent, no matter how small the groups, especially the work of Neefe and Stokes, in which they have reinoculated and remoculated, that I feel relatively happy about those and also about the colossal field study of Gauld in the Mediterranean There it was actually found that the people who had been previously subjected to virus B infection, shall we say, following

protection by gamma globulin  
in experimental and clinical field studies in which gamma globulin has been

B the results have been equivocal In one hospital where the subjects got two inoculations at monthly intervals, it appeared to be effective

Dr R. A. CHANIS (Panama) From a purely clinical point of view, we have been impressed by the fact that in Panama the disease is rather benign, but that very frequently we see that patients come back for years with recurrences, and that sometimes they become chronic patients not many of them, but quite a few—and so I would like to have the speakers discuss that —

Dr W. KAUFMAN (United States) We have heard this afternoon about those two different viruses which apparently are the cause of either infectious hepatitis or serum hepatitis. Now I should like to ask the three main speakers whether there are any evidences in the

logical laboratory tests which have been mentioned this afternoon are in some way reliable in predicting the possibility of the onset of either one of those diseases. One of the discussers mentioned specifically the cephalin flocculation test. In our own limited experience,

whether that test has been of any help.

Dr C. E. VAN ROOYEN (Canada) The jaundice of the horse is not a virus disease, jaundice in the pig is, and jaundice in the rabbit can be produced artificially.

Dr E. G. LEWIS (United States) We are making a study in Massachusetts in New York State, I think the figures are not

York State. But one or two interesting things have come up during the course of this study. One is that among laboratory workers we have had five cases of jaundice, so many that these have become regarded as an industrial hazard. I would be very much interested to know whether similar experiences occurred in other processing laboratories in the handling of wing up of it is almost up of that

type because of the severe risk in patients if they engage in severe with the appropriate tests. At present there are no results from those long term studies.

Dr F. O. MACCALLUM (United Kingdom) Starting with one of the last questions first, I think Dr van Rooyen said that jaundice in the pig had been proven to be a virus. (Dr van Rooyen replied 'be



institution was icterogenic. If only 1 pool that those patients received was icterogenic, then 8 percent of all the plasma dispensed in that hospital was icterogenic. Now, that is potentially a great danger and I think reflects the hazards involved in pooling plasma from even as many as 9 to 22 donors.

In relation to the question of chronic cases and recurrences, I think that everyone is eager these days to find some method of determining residual liver damage and to find how often it does appear. Up to the present there is no good evidence to indicate that it occurs very frequently. I think the best documented group of papers is by Dr. Kunkel of The Rockefeller Hospital. Of 350 patients 23 percent

I think Dr. Neefe will deal  
action tests related to early

van Rooyen brought up the desirability of studying the viruses obtained from patients who had the nonicteric form of hepatitis. I don't know that any of us have had the opportunity to do that, but I think we can say that we have had some experience bearing on the question. The strains that we are using induce in a certain percentage of patients the overt disease with jaundice, and always at the same time and after the same incubation period. A smaller percentage apparently had the same disease and yet failed to develop jaundice. So I think there is no doubt that

inoculated with the same strain of virus, whether or not they develop jaundice. Dr. Makari's concern about the use of cephalin flocculation as a means of determining icterogenic sera deserves comment for several reasons. (1) In studying our volunteers, particularly with infectious hepatitis these tests did not become positive until after the disease was well under way. Now quite in contrast with serum hepatitis, we frequently did obtain positive results with a number of laboratory tests before clinical symptoms appeared but there was no consistency as to which tests would become positive. Another important factor, particularly in respect to the cephalin flocculation test, is the technique. I do not regard it as a simple procedure or an entirely reliable one. I think it is when performed by people who are using it frequently and who are well acquainted with the various technical factors but there are differences in sensitivity of the antigen. The mechanism of the test itself depends on an interrelationship within the serum of the albumin globulin components.

Now as far as other tests go in predicting icterogenic serum I think that is going to prove to be completely unreliable. Certainly, it is pos

In other studies, the results were equivocal in human volunteer experiments, and I feel that this has been due to the fact that this virus is much less common, and that unless you actually study the convalescent serum from actual cases of this type—and I believe this experiment is going on now in this country—until we have the answer on that we won't be sure about the effects of gamma globulin in treating these cases of virus B.

with the test for that purpose and I would remind him that in those instances where there have been most icterogenic pools of serum, no one has ever been able to find the slightest evidence that donors had had any previous illness or infection.

I would just leave one interesting question with you. What pro-

beyond the menopause. This has been up to the present, to my knowledge, an isolated experience in Denmark. Then there was a period in Sweden back in 1931, a rather high mortality rate was reported in a fair sized number of people, without the same age distribution.

In relation to the question about the occurrence of hepatitis in processing laboratories I know of 1 commercial laboratory processing plasma that has had 12 instances of hepatitis among technicians engaged in that work. Those 12 technicians, interestingly enough, had all been treated at an appropriate interval at the dispensary of that institution for lacerations of the fingers. What implications might be drawn from that?

with serum hepatitis. We have recently had occasion to go over the figures of the Pennsylvania Hospital in Philadelphia on the experience there. In this hospital 64 pools of plasma were made over a period of 19 months and given to 621 persons. Of those 621 persons, 6 had, I think, what could be examples of homologous serum hepatitis. That is slightly less than 1 percent, and that in itself seemed very impressive, but it is much more impressive when one realizes that those 6 patients received a total of 10 pools of plasma, so that if all those pools were icterogenic, 16 percent of the plasma of that

institution was icterogenic. If only 1 pool that those patients received was icterogenic, then 8 percent of all the plasma dispensed in that hospital was icterogenic. Now, that is potentially a great danger and I think reflects the hazards involved in pooling plasma from even as many as 9 to 22 donors.

In relation to the question of chronic cases and recurrences, I think that everyone is eager these days to find some method of determining residual liver damage and to find how often it does appear. Up to the present there is no good evidence to indicate that it occurs very frequently. I think the best documented group of papers is by Dr Kunkel of The Rockefeller Hospital. Of 350 patients, 23 percent had evidence of residual liver damage. I think Dr Neeffe will deal with the questions regarding hepatic function tests related to early diagnosis and evidence of hepatic damage.

Dr J R NEEFFE (United States) Dr van Rooyen brought up the desirability of studying the viruses obtained from patients who had the nonicteric form of hepatitis. I don't know that any of us have had the opportunity to do that, but I think we can say that we have had some experience bearing on the question. The strains that we are using induce in a certain percentage of patients the overt disease with jaundice, and always at the same time and after the same incubation period. A smaller percentage apparently had the same disease and yet failed to develop jaundice. So I think there is no doubt that nonicteric hepatitis does occur and perhaps the occurrence of jaundice may at least in part be related to host factors. I am sure that prob-

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able that one may be able to exclude an occasional donor who might be in the incubation period of serum hepatitis, and I think there are several groups who, on the basis of such tests, have excluded a donor and found that after 2 or 3 days the donor did develop hepatitis. So this method perhaps might exclude an occasional person but it obviously wouldn't exclude many others who apparently do contribute virus to pools. As far as the tests are concerned that appear to be the most useful in detecting early hepatitis, we have had to resolve our experience in terms of using a group of tests, and certainly there is no one that consistently is valuable. I think all one can say is that urobilinogenuria is a very sensitive index of hepatic damage, but it also is by the same token one of the most unpredictable and unreliable indications.

enough instances reported to indicate that that may not be a true difference, or at least not a sufficiently consistent one for it to be used as a means of distinguishing between the types of hepatitis. And that relates somewhat to Dr Gear's question concerning the antigen-antibody reaction. We don't know whether, particularly in the chronic cases, the persistent disease is due to virus activity. It certainly behaves much more like a metabolic or antigen-antibody sensitivity, if you will, rather than an infection. At the same time, the whole course of hepatitis, after the first few days, behaves much less like an infectious disease. We did have one experiment where we inoculated a group of volunteers with liver tissue obtained from a volunteer with what was presumed to be a persistent chronic hepatitis. Those volunteers developed a very vague illness after about the right incubation period but they failed to develop any evidence of liver dysfunction, none of them developed jaundice. So I think we are unable to say even whether or not virus still remains. That brings

protect these pools from giving us icterogenic batches of plasma. We have not been able to show the presence of virus in serum collected after hepatitis and protective substances might be present. There hasn't been enough work done on patients who have lingering hepatitis, however, and some of those people might harbor virus.

vent the development of disease by gamma globulin. These attempts have been unsuccessful regardless of whether the globulin is given separately and at intervals, whether it is mixed with the virus and given as a neutralizing mixture, so called, or whether it is given by any other route. So far, it looks as if gamma globulin does not protect against the SH virus of hepatitis.

which might inactivate the icterogenic agent

Dr F O MacCALLUM (United Kingdom) We did an experiment with that. Icterogenic serum was treated by triple ether extraction in the cold and the attack rate was 50 percent before and after

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One point that I wonder about is whether we could not argue the other way, and possibly say that they might be the very ones that would protect these pools from giving us icterogenic batches of plasma. We have not been able to show the presence of virus in serum collected after hepatitis and protective substances might be present. There hasn't been enough work done on patients who have lingering hepatitis,

Depuis la découverte de M Theiler, on savait qu'une immunité solide et durable pouvait être obtenue à partir du virus vaccin neurotrophe de souris

L'application soutenue de 1934 à 1938 en A O F du procédé Sellar Laigret nous avait convaincu de l'efficacité de toute méthode vaccinale utilisant le virus Theiler, mais au point de vue pratique, nous ne pouvions songer à généraliser l'usage du vaccin préparé par Laigret dans l'immense territoire africain, en raison des conditions difficiles exigées pour sa préparation, sa conservation et son inoculation. Tous les efforts de l'Institut Pasteur de Dakar ont tendu à l'établissement d'une méthode simple, applicable dans la totalité de la brousse africaine

Les travaux de mise au point entrepris entre 1938 et 1940 ont abouti au procédé aujourd'hui reconnu par l'organisation mondiale de la Santé sous le nom de "Procédé de l'Institut Pasteur de Dakar"

Le procédé se caractérise

1° — Par la simplicité de la préparation du vaccin à laquelle correspond un prix de revient peu élevé

2° — Par son mode d'inoculation la scarification, qui ne nécessite qu'un minimum de matériel, et de personnel spécialisé

3° — Enfin par la possibilité qu'il offre, par simple mélange de vaccins au moment de l'emploi, de pratiquer en un seul temps la vaccination contre la variole et contre la fièvre jaune. Cette pratique de la vaccination mixte constitue pour les services d'hygiène et de prophylaxie travaillant dans la brousse une simplification considérable de leur tâche déjà très lourde

Nous rappellerons ici

1° — la préparation du vaccin

2° — son mode d'application

*Préparation du vaccin* — Le vaccin est essentiellement constitué par de la poudre de cerveau virulent de souris desséché dans le froid et dans le vide. Le virus employé est le virus de souche française qui est actuellement arrivé au 256-038<sup>e</sup> passage sur cerveau de souris

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Seules, les souris nettement paralysées les 4<sup>ème</sup> et 5<sup>ème</sup> jours après l'inoculation sont sacrifiées

Les cerveaux sont prélevés. Des contrôles de pureté sont pratiqués par ensemencement des aérobioses et anaérobioses

Chaque cerveau est mis dans un petit tube de verre numéroté et placé dans un frigidaire à -25°

Quand tous les cerveaux sont prélevés et congelés, ils sont mis dans une cloche à vide de chlorure de calcium, placée elle-même à l'intérieur du frigidaire à -25°. La cloche communique avec une pompe à huile

## Session 4 YELLOW FEVER DENGUE, AND SANDFLY FEVER

*Friday May 14—9 30 a m to 12 00 m.*

*Auditorium of National Museum*

### VACCIN ANTIAMARIL ET VACCINATIONS ANTIAMARILES ET ANTIVARIOLO AMARILES PAR LA METHODE DAKAROISE EN AFRIQUE OCCIDENTALE FRANCAISE

M PILLET, *Médecin General Inspecteur, Directeur General de la Sante  
Publique de L'Afrique Occidentale Française, Dakar*

La methode de vaccination mixte antivariolo antiamarile de l'Institut Pasteur de Dakar a ete decrite dans une série d'articles et de rapports publies depuis 1939, surtout dans la littérature médicale française mais aussi en 1947 dans le *American Journal of Public Health* qui a bien voulu nous offrir l'avantage de sa grande diffusion.

Le comite d'organisation de notre congres m'a cependant fait l'honneur de me demander de venir exposer cette methode. J'ai repondu avec grand plaisir à cette invitation mais je dois m'excuser à l'avance d'avoir surtout à répéter des choses déjà dites. Je m'efforcerai cependant d'apporter des precisions et des documents additionnels.

L'Afrique Noire Française est dans sa presque totalité située dans la zone d'endémicité amarile. Jusqu'à cette dernière decade le virus amarile manifestait tous les ans sa presence par de nombreux cas mortels reconnus. De nos jours...

La fièvre jaune était la

...t disposer de ressources suffisantes pour entreprendre partout la lutte contre le stegomyia sur d'immenses territoires à population clairsemée. Cette lutte anti-stegomyienne n'est possible que dans les grands centres. Elle est impraticable dans la brousse africaine où même bien appliquée elle ne résoudrait pas le problème en raison de l'existence certaine du virus de la jungle.

Devant les difficultés rencontrées dans la lutte contre les agents transmetteurs nous avons dû nous tourner de plus en plus vers la prophylaxie vaccinale et l'étendre peu à peu à toute la population africaine.



l'intérieur, il est recommandé de mettre le vaccin dès son arrivée et jusqu'au moment de l'emploi dans une armoire frigorifique. De même, les transports du vaccin dans la brousse, pendant la saison torride se font autant que possible dans la glace.

Le vaccin expédié par l'Institut Pasteur de Dakar doit être obligatoirement utilisé dans un délai de deux mois.

Dans la pratique même de la vaccination, le contenu d'une ampoule doit être utilisé le plus vite possible et le vaccin doit être rejeté après une heure de contact avec la température ambiante.

En résumé, nous donnons des instructions pour que la vaccination ne constitue pas un simple geste, mais une mesure prophylactique de valeur.

*Inoculation* — Avec un vaccinostyle on dépose deux gouttes de suspension vaccinale sur la région deltoïdienne. À travers chaque goutte deux scarifications parallèles de 0 cm 5 de long sont pratiquées.

Une surveillance d'environ 5 minutes doit être exercée sur les personnes vaccinées pour éviter qu'elles n'essuient le vaccin ou n'exposent leurs scarifications au soleil. Quand la gomme est bien sèche, la surveillance peut cesser.

On voit donc que l'opération est très simple, réclame un minimum de matériel, et peut-être exécutée dans un minimum de temps. Enfin cette méthode de scarification est en général parfaitement bien acceptée des populations africaines qui redoutent au contraire bien souvent les injections sous-cutanées.

Après la vaccination mixte les réactions locales générales, ainsi que les modifications sérologiques d'immunité présentent les mêmes caractères et s'établissent dans les mêmes conditions que celles qui

in dangereuse

le leur neuro-

tropisme. Cette affirmation peut être faite après un pratique de près de dix ans de la vaccination mixte. D'intéressantes expériences de laboratoires pratiquées par Lépine viennent encore de confirmer également ce point de vue rassurant. Lépine a employé un mélange de vaccin amaril murin et de la neuro vaccine qu'il inocule par voie intracérébrale d'une part à la souris, d'autre part au lapin.

La souris est paralysée vers le 4e ou 5e jour exactement dans les mêmes conditions qu'avec du virus neurotrope amaril simple.

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e exalta

tion des propriétés neurotropes des virus à la suite de leur mélange.

En ce qui concerne les réactions cutanées Lépine, opérant sur le lapin toujours avec le même matériel, aurait noté une légère atténuation des pustules vaccinales après l'usage du vaccin mixte.

Chez l'homme et en particulier chez les primovaccines, nous n'avons pas noté ce phénomène. Les pustules vaccinales se développent dans

qui fait le vide et permet la dessiccation parfaite apres une periode de trois ou quatre jours

Les ampoules sont finement brèves au  
on

Pour un volume determine de poudre de cerveau, on ajoute deux volumes de poudre inerte sterile

Le mélange ainsi obtenu est a nouveau desseche dans le vide a  $-25^{\circ}$  pendant 24 heures. Un deuxième controle de sterilité est pratique. La poudre, reconnue sterile, est alors repartie en ampoules a l'aide d'une cuillère mesure

10

respondant au virus frais au moment

Les ampoules de vaccin, scellées dans le vide, sont conservées a la temperature de  $-4^{\circ}$

La validité du vaccin est de deux mois apres la sortie du laboratoire, a condition qu'il soit conserve en glaciere

Son transport peut se faire a la temperature ordinaire s'il n'excede pas quelques jours

*Mode d'emploi* — Au moment de l'emploi, le contenu d'une ampoule de cent doses est verse dans un tube mortier. On ajoute ensuite goutte a goutte 2 cc. d'une solution de gomme, en remuant constamment l'agitateur

La solution de gomme s'est montée tres supérieure à la glycérine. En effet, la gomme est beaucoup moins fluide elle se desseche rapidement. Au bout de deux ou trois minutes il se forme une petite pellicule qui maintient le virus fixe sur la region scarifiée

La solution employée est la gomme arabique recoltée au Senegal, à saturation, soigneusement neutralisée, filtrée et stérilisée

Pour les vaccinations mixtes, le virus vaccinal anti variolique le plus généralement employé est le vaccin sec préparé par l'Institut de Vaccine de Paris

mêmes que celles recommandées dans la pratique des vaccinations jennériennes dans les régions tropicales. Les campagnes de vaccination doivent avoir lieu, autant que possible, pendant les saisons les moins chaudes, et aux premières heures de la journée et toujours a l'ombre.

11 12 13

se font sans precautions speciales, mais dans les regions chaudes de

Pasteur de Dakar, dans 100% à Montanà et 98.94% à Rio de Janeiro. Les serums de sujets vaccinés avec le mélange variolo amaril ont donné les pourcentages d'immunité positive chiffres à 97.47% à Dakar 98.96% à Montanà et 97.93% à Rio de Janeiro. Ce sont comme on le voit des chiffres tout à fait comparables.

Le troisième point relatif à la durée de l'immunité et à son fléchissement est démontré par les résultats des tests pratiqués sur les populations des villages des environs de Dakar. Sept ans après la vaccination simple 82.4% des sujets sont nettement protégés alors que la vaccination mixte donne 82% de protection dans les mêmes délais.

Les certificats de vaccination ne sont délivrés qu'aux personnes pouvant justifier d'un état civil en règle. Cela veut dire que la

certificats de vaccination qui occasionnellement, leur permettent de bénéficier des avantages accordés aux individus vaccinés. En A. O. F. dans la zone d'endémicité, les porteurs de tels certificats sont dispensés de certaines mesures quaranténaires appliquées dans les localités où apparaît un cas de fièvre jaune.

Ces certificats, du modèle international permettent également de sortir par voie aérienne de la zone d'endémicité. La validité du procédé de vaccination anti-amarile par scarification de l'Institut de la Commission mondiale de la Santé. De tels certificats après vaccination et communiqué.

#### à l'O. M. S.

Les vaccinations mixtes anti-variolo-amariles sont pratiquées en A. O. F. selon un rythme quadriennal. Cependant la régularité de ce cycle a été quelque peu perturbée du fait de la guerre et de l'après-guerre.

Il a parfois été difficile de ravitailler certaines parties des vastes

interprétation des motifs.

est nécessaire dans la

et par nécessité  
minerait obligatoirement  
le 31 Décembre

1949

Le prochain cycle commencera obligatoirement le 1er Janvier 1950 pour se terminer le 31 Décembre 1953 pour l'ensemble de la Fédération.

Voici le détail par année des vaccinations anti-amariles ou anti-

les mêmes délais et évoluent très sensiblement de façon analogue que l'on emploie le vaccin jennérien pur ou mélange avec le vaccin anti-amaril—ce qui a une grande importance dans des régions comme l'Afrique Occidentale Française.

fréquentes; on les observe beaucoup plus rarement chez les sujets de race noire que chez les sujets de race blanche

Chez les Européens, la femme présente des réactions moins fréquentes et moins accusées.

Le mauvais état général des sujets, les affections intercurrentes aiguës ou chroniques, en particulier celles affectant le foie et les reins, constituent au point de vue réaction des conditions favorisantes, faciles à concevoir.

Des milliers d'enfants noirs ont été vaccinés sans accident. Chez l'enfant blanc, on a constaté très exceptionnellement des réactions méningo encéphalitiques graves qui nous ont conduit à recommander

D'une façon générale chez le blanc, et aussi, quoique moins souvent, chez le noir, on peut observer comme après inoculation de tout vaccin anti-amaril neurotrope deux sortes de réactions : celles du 5ème et 6ème jour, et les réactions plus tardives du 12ème et 15ème jour.

Les premières correspondent à l'invasion sanguine du virus amaril; les secondes, à la localisation de ce virus sur le système nerveux.

Les réactions du 5ème et 6ème jour s'observent chez 10 à 15% des sujets. Elles consistent en fièvre, courbature, céphalée plus ou moins vives.

La réaction tardive n'apparaît que chez un petit nombre de sujets. Elle s'accompagne de signes de réactions méningée, et dure en moyenne 5 à 6 jours.

Dans les cas graves, rarissimes, les signes encéphalitiques ou myélitiques dominent la scène. Si aucune médication intempestive n'est instituée, la guérison s'installe peu à peu sans séquelles.

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On trouve confirmation des deux premiers points dans les résultats des tests de séro-protection pratiqués à l'Institut Pasteur de Dakar et en particulier, avec évidence, dans les résultats des expériences

na

pa

anti amaril se sont montrés immunisés dans 96.88% à l'Institut

La valeur de la vaccination est consacrée par l'étude des tests de séro-protection pratiqués, au moins pour les personnes vivant dans la zone d'endémicité amarile, avant et après la vaccination. Plus de 3 000 vérifications ont été ainsi faites, et c'est seulement la pénurie persistante depuis la guerre de matériel indispensable vénéales, thermos, souris blanches, qui nous a empêchés d'étendre ce contrôle absolument indispensable et que nous reprenons de plus en plus.

Nous pouvons dire que des tests ont été faits à peu près avec tous les lots de vaccin, que les résultats obtenus ont été uniformément satisfaisants aussi bien pour les vaccinations faites dans la brousse qu'au centre.

1. Au cours des quatre premières années qui suivent la vaccination, 2 843 sont positifs ce qui donne un pourcentage d'immunité de 95,5

TABLEAU 3 — Les résultats obtenus pour les vaccinations faites

Temps écoulé depuis la vaccination	Nombre de serums contrôlés	Résultat des tests positifs	
		Nombre	Pour-cent
	2 767	2 647	95,6
	139	130	93,5
	54	51	94,4
	16	15	93,75
	134	129	96
	19	18	100,00
Après 7 ans	7	6	85,71
	74	61	82,4
Total ou moyenne —	3 197	3 034	94,9

De la quatrième à la septième année suivant la vaccination, 191 sur 221 tests sont positifs donnant un pourcentage de protection de 86,4

que l'on constate depuis quelques années doit être mise au bénéfice de la vaccination.

Les rares cas observés paraissent avoir pour origine un virus rural, dont l'existence au moins dans certaines régions de la Côte d'Ivoire

ce dernier, au début de la maladie, était négatif. Le cas observé était nettement un cas importé contracté dans la brousse.

En raison précisément de l'existence du virus rural, on ne peut parler de disparition totale du danger amaril en A. O. F., et l'on doit recommander la continuation de la pratique de la vaccination en masse qui nous met à l'abri de toute mauvaise grosse surprise. La méthode de vaccination mixte anti variolo amarile s'est montrée d'ap

variolo amariles pratiquées dans la Fédération pour une population globale d'environ 16 000 000 d'habitants (tableau 1)

TABLEAU 1—*Vaccination anti-amariles ou anti-variolo-amariles*

Années	Vaccinations anti-amariles	Vaccinations anti-variolo-amariles	Total
1939	2 700	98 873	101 573
1940	64 982	23 655	297 637
1941	371 697	1 128 968	1 499 965
1942	281 458	2 885 140	2 866 808
1943	459 339	3 474 893	2 635 222
1944	301 068	3 265 510	3 686 5 6
1945	545 169	2 439 653	2 983 323
1946	309 894	2 307 179	2 611 833
1947	555 609	2 542 222	3 097 831
Totaux	2 982 183	17 011 155	20 653 338

Au 31 Décembre 1947, la population de chacun des territoires de la Fédération était vaccinée dans des proportions qui ressortent du tableau ci après

La population totale de l'Afrique Occidentale Française il semblerait donc que tous des habitants aient au moins été vaccinés une fois. En réalité certains individus ont du être vaccinés plusieurs fois, d'autres ont pu échapper à la vaccination.

On a surtout vacciné dans les régions où la fièvre jaune a prévalu

TABLEAU 2—*Population de chacun des territoires vaccinés*

Territoires	Population (+ ou -)	Total des vaccinations pratiquées
	(1)	336 679
	4 000 000	5 718 836
	1 400 000	2 298 199
	2 100 000	3 036 620
	370 000	119 958
	2 000 000	1 836 558
General	1 700 000	2 680 159
Soudan	2 700 000	3 218 682
Togo	900 000	1 299 258
Réseau du chemin de fer Dakar-Niger		32 309
Totaux	16 320 000	20 633 338

(1) 160 000 habitants (+ une importante population flottante)

## FIELD CONTROL IN YELLOW FEVER

Dr WALDEMAR S ANTUNES, *Director, Yellow Fever Service,  
Rio de Janeiro, Brazil*

Soper has given a masterly definition of yellow fever as "an acute infectious, noncontagious, self limited tropical disease of rapid onset and short duration, due to a specific virus, terminating in death or in spontaneous recovery with the production of lasting immunity"

In severity, yellow fever ranges from an almost inapparent febrile reaction of a few hours' duration to the overwhelming infection and intoxication of the classical case, characterized by albuminuria, jaundice, and haemorrhage

Epidemiologically, yellow fever occurs either as

(1) A domestic human disease with a simple cycle of infection

neither this mosquito nor man is an essential element in the usual vertebrate cycle of infection which maintains the reservoir of virus in the jungle

The basis for the control of any infectious disease can generally be found in a perfect definition of its nature and an adequate knowledge of its epidemiology

Yellow fever, which formerly was a dreaded tropical scourge, today has become a perfectly controllable disease because of unsparring efforts by innumerable scientists

en a system of eradication  
ered the only vector

This was done first

in Habana and later in Rio de Janeiro, to cite only two of the most important areas stricken at that time The results obtained revealed

ce this problem  
without the fear that, as happened in the not too distant past, their cities will become the unnecessary burial places for thousands of human

plication facile, économique Elle est adaptée aux conditions de l'Afrique Son efficacité qui ne fait actuellement pas de doute, sera surveillée par des contrôles périodiques de plus en plus nombreux, au fur et à mesure que nous aurons récupéré les moyens matériels indispensables pour les assurer

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involved, we have but one means to combat the spread of infection—by vaccination. There can be no doubt that vaccination affords the best means of protection, not only for the individual who lives in rural endemic areas, but also for population groups who are in contact with forests where they may become infected, in areas of either endemic or epidemic yellow fever.

The virus currently used in the preparation of vaccine in Brazil is the so called "17D" strain, which has been given in single subcutaneous injections into 4,484,885 persons. This vaccine is manufactured in the laboratory of the Brazilian National Yellow Fever Service. A recent survey of persons vaccinated with this product

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th

economic and practical advantages and has met with considerable success.

Finally, we come to the control of urban yellow fever. In this connection, it may be permissible to mention, without dogmatism but with justifiable satisfaction, the splendid results obtained in Brazil in the largest and most victorious sanitary campaign ever undertaken against any single disease. This campaign was initiated in 1932 under the auspices of the Rockefeller Foundation, which organized the Yellow Fever Service, as it was then designated, on a Nation wide basis.

Experience has amply and repeatedly shown (1) that campaigns directed exclusively at the eradication of *Aedes aegypti* in its aquatic phase succeed in conquering the disease rapidly in cities and towns, and (2) that a really low *Aedes aegypti* index, obtained after 6 to 7 weeks of an antilarval campaign, renders the possibility of autochthonous infection remote.

The preference for humans as a source of the essential blood meal makes this species a domestic vector to such an extent that the experts have considered it most practical and economical to interrupt the mosquito's cycle of development in its aquatic stage. The results have shown the effectiveness of measures put into practice, which were based on the fact that the female mosquito prefers artificial water containers, usually in or near human habitations, for oviposition.

This observation led to the development of the antilarval measure which consists of weekly visits by a sanitary inspector to a given number of buildings in order to search for and systematically destroy all larval and pupal foci of *Aedes aegypti*. Parallel with a progressive fall of the larval index to a constant zero, the inspection cycle is increased to 2, 4, 8, and finally, 52 weeks. Among the advantages obtained by the lengthening of the time cycle is the reduction of

The outlines of these vast campaigns have already been modified and will be further modified as science progresses, either as the study of the behavior of the vector reveals its most vulnerable, as well as its most resistant, biological attributes, or as the brilliant advances of modern chemistry place in our hands substances of increasingly lethal

infected with yellow fever to any part of the world during the incuba

of the insect vertebrate transmission cycle as it occurs in nature has never been attempted because of the obvious impossibility of exterminating the jungle vectors or vertebrate hosts

In order to combat the disease efficiently, it is necessary, in the first place to make known its occurrence in obscure foci. The measure most likely to lead to this objective is the organization of an efficient viscerotomy service with viscerotomy posts as closely spaced as possible in the area under attack. Histopathological examination of liver specimens, obtained through viscerotomy, has permitted the diagnosis not only of yellow fever but also of other diseases of public health importance such as visceral leishmaniasis, schistosomiasis, histoplasmosis, and malaria.

At present, not less than 1,310 viscerotomy posts are functioning in Brazil. Since the inauguration of this service in 1931, a total of 385,728 liver specimens have been obtained from persons who died after illnesses of up to 10 days' duration. Examination of this material has revealed the occurrence of 1,487 cases of yellow fever.

#### MODERN METHODS OF YELLOW FEVER CONTROL

So vital is the viscerotomy service for the discovery of hidden foci of yellow fever infection that omitting its installation is, we venture to say, the simplest way of denying the existence of the disease in any region.

When a case of yellow fever is discovered in a locality infested with *Aedes aegypti*, the patient must be considered to be a possible propagator of the disease. However, if the jungle type of epidemiology is

service was created. In this region periodic dry seasons force the population to store water in large and small containers, generally made of clay, or to keep these containers empty awaiting use whenever rain occurs. These jars are frequently carried by their owners on their forced migrations and many times *aegypti* eggs have been found adhering to the walls of these receptacles. Experiments have shown that these eggs may hatch when dampened by water even though they have been exposed to the sun daily for periods up to 450 days. The reinfestation of numerous localities has been attributed to this circumstance, with adequate justification, and the application of oil has not been effective as a control measure.

The difficulty presented by the resistance of *aegypti* eggs in domestic receptacles constitutes an obstacle of major importance in campaigns of this kind and explains why the eradication program in northeast Brazil has not yet been completed. However, a solution to this problem

started laying eggs located there, or *Apuleia procax* service and the e-

posits, the *Aedes aegypti*, in self defense, returned to its perhaps original habitat to avoid the unfavorable environment created in the vicinity of houses. With the removal of all tree stumps and the filling of all tree cavities with cement or clay, the problem was finally solved.

During the year 1947, no less than 56,414 localities in Brazil were controlled by the National Yellow Fever Service. *Aedes aegypti* were encountered in 28 percent of the localities, but at the end of the year only 13 percent retained a positive *aegypti* index.

#### ERADICATION OF "AÈDES AEGYPTI" AND THE CURRENT USE OF DDT

The concept of eradication was adopted several years ago, long before the use of DDT. The idea that maintaining a low *aegypti* index would offer sufficient protection against epidemic outbreaks of *aegypti* transmitted yellow fever was abandoned. The eradication program became amply justified as it was found easier and cheaper to achieve and maintain a zero *aegypti* index than merely to avoid increases in density of *aegypti* in areas where a low index had been maintained for a long time.

However, eradication of *Aedes aegypti*, which in the Americas is essentially a domestic species and not, as in Africa, a forest mosquito can only be accomplished when a permanent campaign is organized.

If, with methods today considered obsolete, it was possible to eradi-

workers, those released being employed to advantage in adjoining infected areas

Foci of *Aedes aegypti*, however, are encountered not only in buildings but also in various types of river craft where the aegypti may breed either in bilge water or in potable water containers. On some craft, an ingenious tube system has been installed which permits continuous introduction of larvicide to the bilge, even when the vessel is loaded, and so avoids the transportation of mosquitoes to clean areas

The organization of an antilarval service, which is always preceded by a determination of the aegypti index, is no longer based,

It has been repeatedly observed that a low aegypti larval index is not always a definite indication of safety, due to the multiple causes of error which may reduce its value. To ascertain a true index it is necessary to make control captures of adult mosquitoes, and this is of fundamental importance in the anti aegypti campaign. It serves a double purpose. First, it checks the zero index given by the inspector, and second, it furnishes a valuable indication of the presence of breeding foci.

For many years the Yellow Fever Service of Brazil has used oil in the campaign against *Aedes aegypti*. By applying oil systematically to all domestic water containers in contiguous and progressively increasing areas and by policing those areas with squads for the discovery of larvae and of adult mosquitoes, the eradication of *Aedes aegypti* was obtained in the following sections of Brazil: the States of Para, Maranhao, Goiaz, Minas Gerais, Espirito Santo, Rio de Janeiro, the Federal District, Sao Paulo, Mato Grosso, Parana, Santa Catarina, Rio Grande do Sul, the Territories of Acre, Amapa, Rio Branco, Guapore, and Fernando de Noronha, and parts of the States of Amazonas, Piaui, and Bahia.

The total area covered by all the States mentioned is

**Yellow Fever Service.** Of the area where work is being carried out, 87 percent, or approximately 2,000,000 square miles, is already considered clean, that is, aegypti are no longer found. Only 13 percent, or some 300,000 square miles is regarded as infested.

A noteworthy variation in technique was developed in north east Brazil, in areas where a peculiar geo-demographic aspect presents conditions characteristically different from those found in other regions. To meet this problem, a special rural anti aegypti

# PRINCIPAL FACTORS WHICH HAVE ENSURED SUCCESS IN THE ANTI AEGYPTI CAMPAIGN

Among other factors, the importance of the following should be stressed

(1) Passage by the Federal Government, in May 1932, of a law (decree No 23434) granting the Yellow Fever Service authority to employ any sanitary measures deemed necessary in its work. This law has served as a model for those authorizing similar services in other South American countries

(2) Unity of action throughout the whole country in the campaign against *Aedes aegypti*

(3) Extension of the Yellow Fever Service throughout the entire inhabited area of Brazil

(4) The systematic use of oil, both as a larvicide and as a coercive measure

(5) Establishment of breeding foci services

(6) Organization of adult capture services for the continuous verification of clean areas

(7) Compilation of an administrative technical manual for orientation of the staff

(8) Full time service for all employees

inspectors, supervisors, and specially trained doctors

In closing I should like to call to mind the necessity of carrying out the proposal made by the Brazilian delegation at the first meeting of the Council of the Pan American Sanitary Bureau in September 1947, in Buenos Aires. It was suggested at that time that DDT

operating in the final control of yellow fever, which, as Soper has stated, 'is a national problem that requires international action

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has been completed

#### use of DDT

The classic antilarval methods, using oil and a control capture service, were the only methods in use until June 1946, and, as has already been shown, excellent results were obtained by these means. Field experiments on wall surfaces, a method of antimalarial control, found that this system could not be employed economically in anti-aegypti campaigns.

Experience had already shown that aegypti is most vulnerable in its aquatic phase, so that the only necessary change was to substitute DDT for oil. Thus, instead of applying DDT to wall surfaces, it was applied only to the external and internal surfaces of all domestic water containers, whether full or empty, since all such containers must be considered as potential foci.

This method, which is being used in infested rural areas, offers the

overcomes the problem of the resistant eggs, since larvae hatching

The promising results already obtained with this technique make it the most practical and economical method now available for anti-aegypti campaigns. It is hoped that through this means aegypti may be completely eradicated from the northeast of Brazil, its last strong hold in the country.

## THE EPIDEMIOLOGY OF YELLOW FEVER

R. M. TAYLOR and MAX THEILER, *Laboratories of the International Health Division, the Rockefeller Foundation, New York City*

Our knowledge of the epidemiology and prevention of yellow fever has passed through successive phases of groping ignorance, enlightening discovery, high hopes, and partial frustration—all within the last half century

Prior to 1900 nothing was known of the cause or manner of spread of this dread disease which, for more than two centuries, had been a scourge of South American and Caribbean ports and had made periodic seasonal incursions into North America and southern Europe. This was the phase of groping ignorance.

At the turn of the century, Reed, Carroll, Agramonte, and Lazear (1) conclusively demonstrated that the disease was transmitted from

Twenty seven years later the infection was transmitted by the mosquito (2). The work with it under experimental conditions ensued a great surge forward in our knowledge of the properties and behavior of the virus responsible for the infection. This may be termed the phase of enlightening discovery.

As yellow fever was then supposed to be an exclusively human

the New World. This was the phase of high hopes.

It was not until 1900 that it was demonstrated that yellow fever was transmitted by the mosquito. This was the phase of enlightenment. The breaking of the human mosquito cycle was the present phase of partial frustration.

Before summarizing our present concepts of the epidemiology of yellow fever, permit us to recall some of the attributes of the virus which have a bearing upon this subject.

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so successful that the envision  
the American Continent wou  
or the sylvatic reservoir of t  
virus Although *A. aegypti* transmitted yellow fever has become  
rarity on this side of the Atlantic, it should not be forgotten th  
wherever this mosquito exists and there is a possibility that yello  
fever virus may be introduced, the threat of urban epidemics cann  
be ignored This applies not only to the American Continents b  
also to southern Europe, India, and the Far East, where this mosqui  
is prevalent Indeed, it is rather curious, considering the commer  
by ship between Africa and India, that the disease has never invad  
the latter country

In Africa the situation is more complicated, as besides *A. aegypti*  
several other species of *Aedes* may be involved in the transmissio  
of the disease from man to man *Aedes simpsoni* (8) has been i  
criminated and *Aedes vittatus*, *taylori*, and *furcifer* suspected (9)  
These mosquitoes have diverse breeding habits, and they are les  
amenable to antilarval control *A. simpsoni*, for example, is a plai  
axil breeder and is found not only  
ings but also along the edges of fo  
is not so uniquely domestic in it  
Hemisphere, and may be encountered in forests far from huma  
habitations Urban outbreaks of yellow fever still occur in Afric  
and in 1946 there was a rather extensive epidemic involving a numbe  
of large towns and cities in Nigeria (10) Whether or not this an  
similar outbreaks in the past represent extensions from unrecog  
nized endemic urban foci  
from forests is uncertain  
however, that in parts of  
cally initiated by *A. simpsoni*, which, after becoming infected from  
marauding monkeys serves as vector in transmitting the disease from  
man to man

*The forest cycle*—Our knowledge of the propagation and mainte  
nance of the virus in forests is less exact and is probably not complete  
Indeed, when one considers the enormous variety of fauna inhabiting  
tropical forests the task of unraveling the entire natural history of  
the virus in this complicated environment assumes formidable pro

Africa that captured primates frequently show acquired immu  
and in Brazil the virus has been isolated from a species of marmoset  
on four separate occasions (11) It has been shown that the primates  
are, in general, highly susceptible to experimental infection with the  
virus and that the virus may be maintained easily by alternate passage  
through certain species of primates and mosquitoes (7, 12, 13)

more than 5 or 6 days,  
tious to the insect vect  
has ever been observ

immunity associated with the presence of specific humoral antibodies

When an appropriate mosquito imbibes blood containing the virus the virus proceeds to multiply (5) in the body of the mosquito, and after a period of 10 days or more, dependent upon the ambient temperature (6, 7), the mosquito is able to transmit the infection by bite to a susceptible animal. The mosquito retains and is able to transmit the virus throughout its natural life. The longevity of the mosquito is not affected by the presence of the virus. Thus from the standpoint of time, the mosquito constitutes a more permanent reservoir of the virus than does the vertebrate host. However, no transovarian passage in the mosquito has ever been observed and the sojourn of the virus in the vector is thus limited to the life of the infected mosquito. Since the virus is constantly linked to either host or vector, it is obvious that its natural history and the epidemiology of the disease it produces in man and animals is dependent upon the species of the vertebrate hosts and insect vectors involved in its cyclic passage.

Two epidemiological patterns of the disease are recognized: the

plateau area in South America and the Caribbean area and for the periodic seasonal excursions of the disease to North American and southern European ports. The course of urban yellow fever is conditioned by the size and stability of the human population involved and the prevalence of the mosquito vector. In general the virus can be maintained in endemic form only in large human aggregates where the newborn, after loss of their initial maternal immunity, together with transients, furnish a constant and adequate supply of susceptible hosts or where there is a shifting population accompanied

reservoir cannot be denied. This hypothesis has been entertained by some because it is felt that the monkey mosquito cycle hangs by too delicate a thread to explain persistence of the virus in endemic form and, above all, to account for the rapidity of spread and the seasonal character of the epidemic excursions in southern Brazil.

Certainly, if the virus remained in a restricted area for a long period, the monkey mosquito cycle would not offer an acceptable explanation, as the surviving population of monkeys would soon become immune,

and is thus endemic in the extensive tropical rain forests, by virtue of wandering epidemics, or rather epizootics.

The rapidity of extension of the epidemic thrusts from forest to forest in subtropical Brazil cannot be satisfactorily explained by the migration of primates, nor does it appear that man is responsible, except rarely, for carrying the virus from forest to forest. Recently it has been demonstrated that forest mosquitoes may fly or be carried by the wind for several kilometers over open country from one forest patch to another (20). This may account for the spread of these epidemics, but we are still in the dark on how the virus survives through the cold season when the known mosquito vectors virtually disappear.

By means of immunity tests in the human populations and in captured primates and the examination of liver specimens of persons

the extent of the yellow fever virus has been  
 endemic in  
 the Orinoco  
 in Brazil  
 Amazon

#### Expert Commission on Quara

excursions of the virus, engulfing forested areas in southern Brazil and Paraguay, have periodically occurred. These epidemic waves, which appear to result from spill overs of the virus from the northern

Although man is not directly involved in the jungle cycle, he

As for vectors, the only positive evidence relates to mosquitoes. A number of species of mosquitoes belonging mainly to the genera *Aedes* and *Haemagogus* have been found capable of transmitting the virus by bite. In South America the virus has been isolated from captured mosquitoes of the genus *Haemagogus* on 18 occasions, three times from *Aedes leucocelaenus* and once from Sabethine mosquitoes. In Africa the virus has been found in *Aedes simpsoni* and once in *Aedes africanus* (14) which on ecological grounds (15) is thought to be an important vector in certain African forests.

In addition there is circumstantial or indirect evidence which points to the involvement of primates and haemagogus mosquitoes in the forest cycle of the virus in South America. There is a correlation between immunity in primates, the prevalence of haemagogus mosquitoes, and the immunity in persons who have contact with the forests. The type of forests in which the virus is found is also the type which furnishes a favorable habitat for primates and haemagogus

South America and *A. africanus* in Africa find their most favorable environment in the canopy of the forests as do also the primates, and it is therefore presumed that most of the transmission occurs

Haemagogus mosquitoes feed during the day, while *A. africanus* is a crepuscular or nocturnal species. This has a bearing upon human infections.

The search for other hosts and vectors has led to negative or inconclusive results. Some of the marsupials are relatively susceptible to infection with the virus (17-19), but the species which have been found susceptible in the laboratory are apparently rarely infected in nature. Conversely the species which have shown some evidence of having acquired immunity in nature are rather resistant to experimental infections. It may be here remarked that quite a variety of animals may be infected with yellow fever virus to the extent of developing specific antibodies and occasionally circulating small amounts of virus but are incapable of infecting the known mosquito vectors. Likewise other genera of mosquitoes besides those men-

tioned but these have no part in the propagation or maintenance of the virus in nature. If these dead end infections are disregarded then the only proven vectors of the virus are mosquitoes of the genera *Aedes* and *Haemagogus* and the only proven animal hosts are primates.

However, the possibility that the virus may exist in the forests in some masked form and that the monkey mosquito cycle is only a periodic and secondary manifestation stemming from some underlying

as man rarely remains in the forest after nightfall, yellow fever is infrequently contracted by forest contact

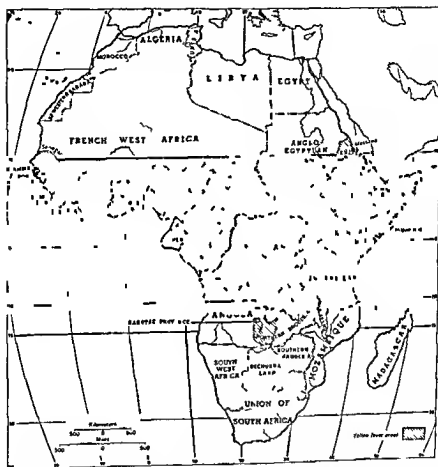


Figure 2.—African yellow fever area delineated by Expert Commission on Quarantine

When a man becomes infected by *sylvan acgypti* is present in the epidemic may ensue In

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#### IV VIRUS AND RICKETTSIAL DISEASES

With the suppression and, indeed, the eradication of *A. aegypti* in wide areas in South America, practically all human infections in recent years have arisen from forest contact. The infection rate of jungle-acquired yellow fever is significantly higher in males over 15 years

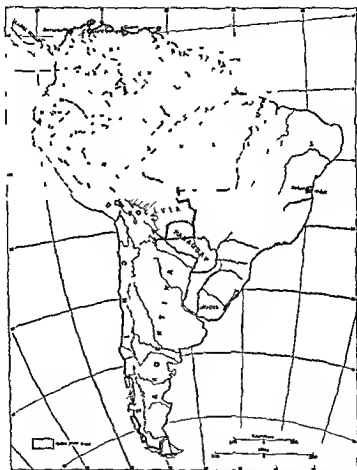


Figure 1.—South American yellow fever area delineated by Expert Committee on Quarantine

of age, as they more frequently enter forests than do women and children. This is in sharp contrast to the age and sex distribution of the urban or man-mosquito-man form of the disease, which attacks all ages and both sexes with a preference for the stay-at-homes or women and children. It has been mentioned that in Africa *A. africanus*, one of the supposed man-jungle vectors of the virus, is a night feeder,



to man. In West Africa, where *A. aegypti* is the principal urban vector, epidemics in towns and villages are commonly associated with infection of primates in the neighboring forests, but the frequency and exact manner of exchange of the virus between forest and village is not known.

The origin of yellow fever virus is a matter of speculation, but it seems not improbable that it first arose in the fauna of tropical forests and that the disease in man represents a secondary offshoot. Since the virus strains isolated in Africa and South America are virtually identical, it is reasonable to assume that they had a common origin. Whether the birthplace was in Africa or South America may ever remain in doubt, but it is reasonably certain that *A. aegypti* is an Old World species and that urban epidemics of yellow fever in the Americas date from the introduction of this mosquito.

## SOUTH AMERICA

TOWN

JUNGLE

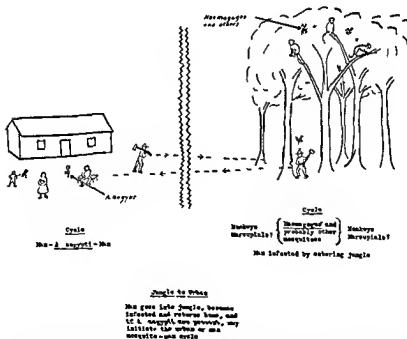


Figure 3.—Transmission by *A. aegypti* is represented as occurring outside of the house, while in reality it is probable that most of the transmission occurs within doors. The broken lines indicate that the house may be some distance from the forest.



of mosquitoes have been consistently taken in significant numbers

found naturally infected with yellow fever virus have been *Haemagogus spegazzini*, *Haemagogus capricornu* and *Aedes leucocelaenus*

Furthermore the groups of forest animals which when bitten by infected insects circulate enough virus to infect other insects, are limited to the order of primates and to certain species of small opossums. There are indications, therefore, that the sylvan problem may not be as complex as was originally supposed. Certainly the epidemiology of urban yellow fever is simple enough. It requires but two components, man and *Aedes aegypti*

more important in this respect than movement of monkeys. Stained more than ten

first like to congratulate Drs Taylor and Theiler on the clear and concise manner in which they have set forth the present conception of the epidemiology of yellow fever. As it has not been possible for them, in the time at their disposal, to cover in detail much of the work on which their conclusions are based, I propose to add a few comments on some of the recent studies which have been made in Africa

Our understanding of the epidemiology of yellow fever in Africa is based on work which has been done by the staff of the Yellow Fever Research Institute in Uganda, in an area in that territory known as Bwamba County. This is a small heavily forested area which lies in the extreme west of Uganda between the Ruwenzori Mountain and the Semliki River, on the Uganda Congo border. The northern portion of the county is occupied by the uninhabited Semliki Forest, an eastward extension of the great rain forest of the Congo. Between this forest and the mountain, there is a cultivated area with a population of about 35,000. An intensive survey of these people showed that, while immunity to yellow fever among adults was widespread, the percentage of immunes rose steadily as the un-  
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population was carried out, but the mosquito studies were continued

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#### ABSTRACT OF DISCUSSION OF PAPERS BY ANTUNES AND TAYLOR AND THEILER

Dr HENRY W KUMM (Brazil), commentator In his comprehensive paper Dr Waldemar Antunes has emphasized the three main foundations of the yellow fever control service of Brazil These are, eradica-

tensive mosquito control carried out in the big cities alone brought about the termination of yellow fever Thus the "key center theory" of *Aedes aegypti* control arose and it prevailed until the early 1930's In Brazil however, key center control failed to eliminate the disease Two of the factors responsible were first, the occurrence in the dry northeast of this country of intense *Aedes aegypti* breeding in urban and rural areas alike, and, second, the existence of endemic jungle yellow fever over wide areas of South America

populated

Southern States, not only destroys forever the natural habitat of wild monkeys but also the home of the very sylvan mosquitoes, which are responsible for transmitting that disease from monkeys to men

At first sight the epidemiology of jungle yellow fever might appear to be very complicated, because of the immense variety of biting insects and wild animals that abound in the forests of South America Actually, however, whenever extensive captures of insects have been made in areas in which sylvan yellow fever has prevailed, only certain species

it is essentially a disease of monkeys which is introduced from time to time into areas inhabited by man

Dr KENNETH SMITHURST (Uganda). I can't do anything but compliment the speakers on what has already been said, but I should like to make a few brief remarks about some very recent work in Uganda. Within the past 2 years we have been making a survey over the country to try to correlate information obtained in the forest areas with

Within the forests, *Aedes africanus* is present up to, but not exceeding, five thousand feet altitude, and in the plantations near the forests, So we

any testimony on the experiments that have been made in Brazil in the south of the State of Minas Gerais by Dr Kumm. It seems to me that these experiments are of the utmost importance. We can now understand that monkeys do not play an important part in the passage of the virus from one jungle to another. Instead of monkeys, we are quite sure now that this role is played by mosquitoes. Mosquitoes may fly as far as 6, 8, or 10 kilometers, principally as an effect of the predominant direction of the wind. Maybe this can help us to understand the direction which the virus is taking when it is spread over a certain zone.

problem in Africa and in the American continent. I understand quite well that we could not adopt identical measures in Africa and the American continents. Besides, we know quite well that in the American urban yellow fever is carried by the domestic mosquito, *Aedes*

seems to me that the only means we have against jungle yellow fever is to try to vaccinate people who live in the jungle or near the jungle, or who have to pass alongside the jungle, also the Army troops, because we never know when a regiment will receive orders to go through the

both in the populated area and in the forest. Yellow fever virus was

human population was known to be immune at the time of these isolations, they indicated a persistence of the virus in a vertebrate host other than man, presumably in the wild animals of the forest. A study of these animals was, therefore, commenced and carried on simultaneously with the mosquito work.

During the course of the animal studies, a large number of blood specimens was collected from many different species and examined in the protection test, but immunity to yellow fever was found among monkeys only. In these animals, of which at least 12 species are represented, immunity is widespread. Furthermore, the incidence of immunity increases with increasing age, in all species, and evi-

the ground, showed a high incidence of immunity indicated that a vector with arboreal habits must play a part in the transmission of the infection. A study of the arboreal mosquito fauna of Bwamba was therefore necessary.

To gain information on the vertical distribution of mosquitoes in the forest, a long series of 24 hour catches was carried out simultaneously at ground level and on tree-platforms of various heights, up to 52 feet. Human bait was used, and the catch at each station for each hour of the day and night was recorded separately. The results provided a mass of information on the biting habits of the

transmission of yellow fever from monkey to monkey in the Semliki Forest, and it may well have equal importance in other forested areas in Africa.

To sum up, then, we have in Bwamba evidence of a man to man yellow fever cycle —  
to monkey cycle tr.

*Aedes africanus* is

that yellow fever is endemic in the monkeys of the forest, and that

# RECENT ADVANCES IN PHLEBOTOMUS AND DENGUE FEVERS

ALBERT B SABIN, M D, *The Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati Ohio, United States Army Epidemiological Board*<sup>1</sup>

Phlebotomus (pappataci or sandfly) and dengue fevers, two insect borne virus diseases which are endemic in certain parts of the world became a problem to the American armed forces during World War II when thousands of individuals acquired these illnesses and were temporarily removed from duty, frequently at a time when their services were badly needed. The importation of dengue infection into parts of the world ordinarily free from the disease but harboring the mosquito vectors became a vivid possibility with the Hawaiian

and adjacent Oshka between 1942 and 1943. Immunizing agents against these diseases made it necessary that we learn more their for p

phlebotomus and dengue fevers could not be taken off somebody's shelves or refrigerators and be submitted to study. They first of all had to be recovered and identified from among the many febrile illnesses which were occurring in the Mediterranean and Pacific areas and thus could not be done without the generous participation of American human volunteers. The few advances which have been made in the studies which were begun in 1943 and are still being

h serum from patients with etiology, only phlebotomus

of these viruses were recovered from the Mediterranean area and only

be able to demonstrate in human beings in the United States and thus not exposed to the virus except under ex

<sup>1</sup> - active duty serving with the epidemiological Board Office of The while working with the aid of

jungle in certain areas which may be just the place where jungle fever exists.

Dr JOHN A. KERR (United States) First of all, I wish to say that  
 5 years  
 of DDT  
 entirely

In 1944 it was my good fortune to be in Italy where there were huge numbers of *Anopheles* in houses and stables near areas which had been

critical 10 percent of the inside walled surfaces of those houses to be treated, a 10 percent which would kill 90 percent of the *Anopheles*

most beautiful demonstration of applying the minimum amount of DDT and obtaining at the same time maximum effects I think Dr Antunes and the service he is directing are to be most highly complimented for working out this excellent procedure

was transmitted by small numbers of *Aedes aegypti* mosquitoes after an appropriate extrinsic incubation period and by establishing for the first time that the particle size of the virus, as measured by gradocol membrane filtration, was in the range of 15m $\mu$  to 22m $\mu$ . These properties clearly differentiated dengue virus from the phlebotomus fever group and pointed to a certain kinship with the virus of yellow fever, which greatly influenced the design of all the subsequent work.

In dengue, also, there was no satisfactory information on the immunity which followed a single infection, and the not uncommon histories of repeated attacks under natural conditions, taken together with the inability of former investigators to demonstrate protective antibodies in the serum of recovered individuals, led many to assume

ever, it was found that there were multiple immunological types of virus, that resistance to the homolog (at least 18 months thus far), and that antibodies could be demonstrated by appropriate concentrations (not more than 1,000 infective doses) of virus were used. These tests in human volunteers were further facilitated by the discovery that dengue virus produced local skin lesions within a few days after intracutaneous injection, and that these could

This group includes  
her types of den-  
us fever virus or  
embryo vaccine

nesses of 1 to 3 days' duration, clinically not recognizable as dengue, but which, nevertheless, were proved to be dengue by both mosquito and blood transmission tests. It is of interest in this respect, that

in 1944 there were many cases of 3 days' duration but which yielded typical dengue virus in human volunteers in the United States. It is furthermore, noteworthy that among the four strains of virus recovered from New Guinea, the Hawaiian strain, while the group fashion previously

Two strains of virus recovered in 1945 from Americans in

perimental conditions, that complete immunity was present at 2, 4, and even 24 months after a single attack, provided the same strain of virus was used for reinfection (1, 2). When the second strain of virus was recovered from Americans in Sicily, it was found to be immunologically identical with that from the Middle East, even in cross immunity tests carried out after an interval of 2 years (2). However, in 1944 another virus was recovered from Americans in the Naples

teers who had been proved immune to the Naples virus. Similarly, volunteers who had recovered from infection with the Middle East Sicilian virus had no immunity to the Naples virus (2). Yet there is every reason to believe that the Naples virus is a true phlebotomus fever virus even though there has not yet been an opportunity to test the capacity of *Phlebotomus papatasi* to transmit it. The reasons are as follows: (1) The experimental disease in volunteers is the same as

whether or not there are more, one cannot say.

*Phlebotomus* is the only vector known to transmit the virus.

such a broad range can hardly be regarded as coincidental.

Another strain of virus (2) also was carried out on human volunteers in the United States, and seven strains of virus, recovered from Americans who had illnesses of varying severity in Hawaii, New Guinea, and India, were subjected to detailed study and immunologic analysis. The first strain of virus recovered from Hawaii early in 1944 was completely identified, not only by the reproduction of clinically typical and severe dengue, but also by proving that it



that were tested. Two week old or younger mice adapted after many serial passages in young mice, would succumb with regularity. The diagram in chart (fig 1) shows only that portion of the passages which in the early passages exhibited nothing or further passage. Only 10 to 20 percent of the at first exhibited clinical signs of the infection (slight of the extremities detectable only by special tests in some, paralysis or encephalitic signs in others), and the period was frequently 3 to 4 weeks. It took 15 passages

the period until now, after more than 80 such passages, the cerebral titer for the 0.03 cubic centimeters dose in mice is 10<sup>6</sup>, and the incubation period for the highest concentration is approximately 6 days. We could not be certain that this virus in was indeed dengue virus, until, after appropriate preliminary in laboratory animals, the early passage material was inoculated in human volunteers and produced in them solid immunity to unmodified human dengue virus. Similarly, we know that the virus

adapted dengue virus produces neither apparent nor inapparent infection in cotton rats, hamsters, guinea pigs, or rabbits.

Since the end of the war we have discovered that the three strains of dengue virus propagated in mice by Drs Hotta and Kimura in Kyoto, Japan, had the same biological and immunological properties as ours, while two other strains reported by other Japanese investigators as mouse adapted dengue viruses were identified in one instance as Rift Valley fever virus and in another as fixed rabies virus.

illness and protracted fever characteristic of the original disease but retained the capacity to produce the rash (6). Thirty three human volunteers inoculated with varying quantities of mouse brain extract containing this modified virus proved to be solidly immune to infection with the unmodified virus and demonstrated the feasibility of using this material as a vaccine. It has also been shown that

of virus was probably responsible for the bulk of the dengue in Japan. On the other hand, neutralization tests on sera obtained from American marines who had had the disease on Guam and from Americans and Panamanians in the Panama Canal Zone, indicated that other types of dengue were probably more prevalent there.

Many different and unsuccessful attempts to prepare a practically useful immunizing agent were made before the modified or mutant strain of mouse adapted virus was developed. Thus, serum containing approximately 1,000,000 human infective doses per cubic centimeter produced no immunity after the virus had been inactivated by ultraviolet light under optimum conditions. Extracts of infected mosquitoes, similarly inactivated by ultraviolet irradiation or by formalin, were also without immunizing capacity. Amounts of dengue virus which just failed to produce clinical evidence of infection did not produce complete immunity and were impractical for other reasons as well. Attempts to produce immunity without disease by introducing the virus by unusual routes led to the discovery that dengue could be produced by merely rubbing the virus into the scarified skin or instilling it into the conjunctival sac or into the nasal passages, although the latter procedure produced in the majority of volunteers a very mild and modified form of the disease. In this connection it was also found that the modified yellow fever virus contained

warrant use of this procedure for immunization against dengue.

Exhaustive attempts to grow the human dengue virus in embryonated eggs and in a variety of tissue culture media were unsuccessful. Similarly, there was no clinical evidence of infection in many tests on mice, hamsters, cotton rats, guinea pigs, and rabbits with virus of proved high potency for human beings. Inapparent infection, at the time demonstrable only by passage to human beings, occurred in rhesus monkeys. However, the many similarities between the viruses of yellow fever and dengue and the available knowledge of the varying behavior of yellow fever virus in mice, were, in large measure, responsible for the persistence with which my associate, Dr

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 factor apparently present in infectious human serum. The best re

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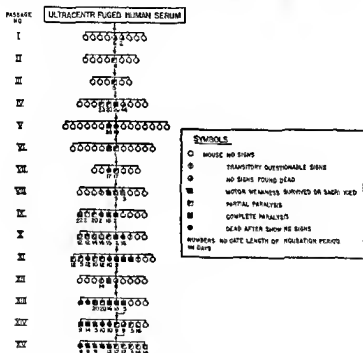
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mosquitoes feeding on vaccinated individuals do not transmit the disease. After the virus was thoroughly adapted in mice, it proved

when the potency of the virus in mice was only one thousandth of what it is now. Serological tests with the mouse adapted virus have been developed and have already found a certain usefulness in both diagnostic and epidemiological investigations.

## ADAPTATION OF DENGUE VIRUS (HAWAII STRAIN) TO SWISS ALBINO MICE



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- (5) Sabin
- (6) Sabin

has been the destruction of and the protection against the vector the *Aedes*

Vigorous measures, including detection and elimination of breeding places spraying of hiding places of adult mosquitoes, segregation of dengue patients in screened camps in an area free from *Aedes*, application of a repellent to the exposed parts of the body, wearing of long sleeved shirts and full length trousers, have proved to be effective in the control of dengue under war conditions (41, 56) Among the insecticides DDT plays a prominent part in *Aedes* control

The therapy being symptomatic, no special drug has been recom

Le Gac and Servant (18) recommend lumbar puncture to relieve the severe headache, the stiffness of the neck, the pain in the back and the low pulse by decreasing the hypertension of the cerebrospinal fluid

I now come to the question of the homogeneity of the group of so

has been  
r may be  
considered as a separate disease entity on account of its distinct clinical  
ological difference

igators (45, 46)  
A strain of virus which was frequently passed through patients in mental hospitals for the treatment of schizophrenia was cultivated on the allantoic membrane of the growing chick embryo At the site of the

the successful propagation of the sandfly fever virus on the chick

nature  
its ob-  
tained infecting mice and monkeys by the intracerebral method On the contrary, Shortt, Poole, and Stephens (79) were able to infect healthy monkeys intracerebrally with sandfly fever virus and to produce the disease in a  
of the sick monkeys

Sabin, Philip, and

Africa

ralla (77) There were many complications corneal ulceration, meningismus, postfebrile neuralgia, otitis media, and acute gastritis The death rate was about 1 percent.

In the cerebrospinal fluid there may be some increase of albumen content and considerable increase of the sugar content (18), and in some of the fatal cases during the great epidemic in Athens in 1930 there were post mortem findings of encephalitis (28)

As for the transmission of dengue, thanks to the fine experiments of Mackerras et al (7), a third *Aedes* species has joined the well known vectors *A. aegypti* Linn and *A. albopictus* Skuse, i e, the *Aedes scutellaris* Walker subspec *hebrideus* Edw in New Guinea

On epidemiological grounds, *A. taeniorhynchus* has been suggested as a vector in Florida and *A. albopictus* var *hebrideus* as a vector in

Coles (11-22) described granular bodies in red cells in blood smears of dengue patients measuring  $0.25\mu$ - $0.4\mu$ , which he called *Maculae dengue*

Very important progress was made by the experiments of Sabin and Schlesinger (62), who adapted the dengue virus to mice by the intracerebral route Most of the experiments were carried out with a particularly virulent strain from Hawaii which showed a very high concentration in the serum of experimentally infected volunteers The size of the virus was about  $20m\mu$ .

Comprehensive attempts have been made, even by ultracentrifugating highly concentrated preparations, to cultivate the virus in media containing mouse embryo tissues or to infect developing chick embryos All gave negative results The King Institute in Madras, however, has claimed the cultivation of the dengue virus on the

for the initial infections In later passages the virus became gradually adapted to mice, and in the fifteenth passage all of ten 3 week old mice developed signs in the central nervous system

The mouse passaged virus was not pathogenic for cotton rats, hamsters, guinea pigs, or rabbits After the seventh mouse passage, the pathogenity for man had so decreased that immunization could be induced almost without any signs of disease, especially when the virus was injected in combination with yellow fever vaccine Bites of *Aedes aegypti* infected with the modified virus also produced immunity in volunteers.

The modified virus therefore, might be used as a vaccine in the control of dengue fever (75) Until now the only means of control

The handicap in virus research is the difficulty of investigating the properties of a given virus without altering it. The adoption of

virus. The transmissibility also may be changed.

Luckily there is one property that remains constant even after adaptation, i. e., the antigenic pattern. In comparing different viruses therefore, we can make good use of tests based on antigenic properties such as cross immunity, cross protection and neutralization tests and cross complement fixation tests, using suitable concentrated antigens of the viruses.

In recent years thorough investigations have put two new disease entities next to dengue and sandfly fever in the group of dengue like fevers. These two new entities are Bullis fever and Colorado tick fever.

Bullis fever was first described by Woodland, McDowell, and Richards (33) as a new disease, clinically almost similar to dengue occurring in 1942 among soldiers at Camp Bullis near Houston, Tex. The evidence of a tick borne disease was highly suggestive, all the patients having been bitten by *Amblyomma americanum* shortly before onset.

Livesay and Pollard (34) infected guinea pigs with the blood of patients by the intracerebral route, and Anigstein and Bader (33, 36) could establish an infectious agent in guinea pigs from a collection of 500 *Amblyomma americanum*, which afterwards (35) could be identified with the virus of Livesay and Pollard. As all these investigators described the causal agent as rickettsiae, which were demonstrated in the blood, peritoneal fluid, and in the organs of the guinea pigs and in the blood of the patients, regarded as being like those of rickettsiae after passages in guinea pigs and in the blood of the patients, which caused scrotal swelling with the human and which showed the typical

### Bullis fever (37)

The virus has been passed through mice, and from the second passage it has been cultivated in the yolk sac of 5 to 6 day old chick embryos. With yolk sac material of the sixth to twentieth passage, volunteers were successfully inoculated (73).

There was no cross immunity in guinea pigs between Bullis fever and Rocky Mountain spotted fever, typhus, Q fever, scrub typhus, Chagas's disease, equine encephalomyelitis, and lymphocytic chorio meningitis (34, 55).

in 1943. The virus was found in the blood of patients, though never later than 48 hours after onset of the disease. No virus could be demonstrated in the cerebrospinal fluid. The size of the virus was esti-

to volunteers by *Phlebotomus papatasi* reared in the laboratory, control tests with *Aedes aegypti*, *Culex pipiens* and *Pulex irritans* gave negative results. Immunity of short duration could be induced in volunteers by experimental infections and even without clinical re-

passing viruses. Now I should like to emphasize the fact that the clinical picture alone is certainly not sufficient to decide whether

different clinical course. Among these are pretibial fever among

(84), "seven days fever" (22, 23), seller fever in northern India (29, 30), Bessarabia fever (26, 31), and Russian headache fever (31, 32, 66), which differed from dengue by the occurrence of "meningeal" attacks in some of the cases.

As it was found that the same organism was the cause of these diseases, it was not surprising that the same vector, *Aedes aegypti*, was found to transmit them. In dengue there may be found other vectors than *Aedes*, but a disease of humans which cannot be transmitted by *A. aegypti* is not true dengue.



logical individuality of Colorado tick fever virus. Cotton rats and opossums did not succumb to infection as did hamsters and mice, but the virus did circulate in their blood, sheep and rabbits were not susceptible. T

Afterward

yolk sac of

virulent for mice by the intracerebral route but not any longer for young mice and hamsters by the intraperitoneal route. From the thirty seventh egg passage, volunteers could be infected, who showed only minor clinical symptoms. In their serum, specific neutralizing

*andersoni* and distinct from dengue and Bullis fever. It differs from dengue by its clinical course and its epidemiology. The hamster is susceptible to Colorado tick fever virus but not to dengue virus.

In human volunteers, there is no cross immunity between Colorado tick fever and dengue (72, 76) or between Colorado tick fever and Bullis fever (73).

We may state that a great deal of work has been done in the last years which has deepened our insight into the group of dengue like fevers. We are thankful, it is true, but not satisfied yet. There are many questions still unanswered. The comparative study of dengue and Bullis fever virus, which have both been adapted to the same animal, the mouse, should be intensified. The experimental transmission of Bullis fever by its presumable vector has not yet been accomplished. Whether Bullis fever and Colorado tick fever virus can be transmitted by *Aedes* and whether ticks can be a vector for dengue virus have not been investigated yet.

Of the clinical syndromes claimed as new disease entities within the dengue group, it should be established how they are transmitted. Their immunological relationship to dengue and other approved entities of this group will have to be taken up.

There is only one more point to which I should like to draw your attention, i. e., the problem why yellow fever did not penetrate into vast tropical regions where *Aedes aegypti* are abundant. About 18 years ago, we produced in Amsterdam some experimental evidence (86, 87, 88) that dengue infection could give rise to a partial immunity against yellow fever in monkeys. The question of the possible immunological relationship between these diseases might be taken up again, with the mouse as susceptible animal to both viruses.

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In a later communication Howard et al (43) concluded that the causal agent "appears to approximate in size the elementary body agents such as ornithosis more than it does typical rickettsia." Volunteers could be infected with Seltz filtrates of serum or with blood of patients. Perhaps the filter-passing agent isolated by Steinhilber and Parker (54) from rabbit ticks in Camp Bullis may be the same virus. Whereas, in all naturally and experimentally infected human beings and animals the Weil-Felix reaction was negative (36, 51), it may be regarded as highly improbable that Bullis fever belongs to the typhus group.

We can conclude that Bullis fever is a dengue-like disease caused by a filter-passing virus probably transmitted by the *Amblyomma americanum*. In the group of dengue-like fevers, it can be considered as a distinct disease entity. In volunteers, there was no cross-immunity between Bullis fever and dengue (2) or between Bullis fever and Colorado tick fever (73). The animal reservoir is still unknown, sera from 4 of 40 deer (*Odocoileus virginianus*) shot in the camp area and from 2 of 7 rabbits (*Lepus californicus*) were positive for Bullis fever (51), a strain of Bullis fever virus could be isolated from a pooled suspension of *Amblyomma americanum* collected from a deer in the area (73).

succeed (50).

Recently Florio and Miller (88) isolated the virus from several pools of *Dermacentor andersoni* obtained from areas where individuals had presumably acquired the disease.

The infection in the tick is transmitted to progeny. Moreover dog ticks (*D. variabilis*) obtained from Long Island have been found infected.

Florio et al (50) found the hamster susceptible, volunteers could be infected with the virus from the hamster.

gated already in 50 mouse brain passages. Young mice 8 days old could also be infected.



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## Session 5. TROPICAL POLIOMYELITIS

*Saturday, May 15—9 30 a m to 12 00 m*

*Departmental Auditorium, Room B*

The following resolution was presented by Dr Felix (United Kingdom) and seconded by Dr Castañeda (Mexico) and adopted

The Section on Virus and Rickettsial Diseases is of the opinion that there is great need for standardization of the materials and methods employed in the serological tests used in the routine diagnosis of the rickettsial diseases. The Section recommends that the World Health Organization take the appropriate steps to insure the adoption of an internationally agreed standardized technique

### THE GEOGRAPHICAL INCIDENCE OF POLIOMYELITIS WITH SPECIAL REFERENCE TO SOME FEATURES OF THE DISEASE IN THE TROPICS

A J RHODES, M D, F R C P, L D S C, *Research Associate, Connaught Medical Research Laboratories, and Associate in Virus Infection, School of Hygiene, University of Toronto, Canada Formerly Lecturer in Bacteriology, London School of Hygiene and Tropical Medicine (University of London), England*

#### INTRODUCTION

In the past 5 or 10 years, however, the subject has come into prominence for two main reasons

(1) The disease proved unexpectedly common in British, American and other Allied troops serving in the Middle East, India, the Philippines, China, and Japan (van Rooyen and Morgan, 1943, Paul Havens, and van Rooyen, 1944, Illingworth, 1945, McAlpine, 1945, Coughy and Porteous, 1946, van Rooyen and Kirk, 1946, Sabin, 1947). In fact, it was estimated that the incidence in these theatres was about 10 times that in home commands. These troops served as human guinea pigs and drew attention to the presence of poliomyelitis virus in communities where the disease did not appear to be prevalent in the native population at the time

ever, the two viruses interfere one with the other, as I have indicated, not only in human beings but also in monkeys. I would like at this time to mention briefly experiments which were carried out in association with Dr. Max Theiler. These experiments were as follows. It is known that mosquitoes that are infected with yellow fever remain infected for life. It is also known that mosquitoes infected with

that was presented to a mosquito that had dengue in it was not too

epidemiological phenomenon, because if that were the explanation, it would be very odd indeed. But it is interesting to keep this in mind.

lation of monkeys Even if virus is isolated from stool or nasopharynx, this does not necessarily denote that a nervous illness is due to poliomyelitis, for healthy carriers are not uncommon So few strains have been adapted to rodents, that there is little justification for using these animals for routine isolations Serum neutralization tests with a rodent adapted virus as antigen have not proved of value in diagnosis

There is scope for much further work to be carried out on strains of poliomyelitis virus occurring in the tropics, where the supply of monkeys should not present the problem it does to workers in North America and Europe In particular, an urgent problem is the study of the antigenic structure of strains collected from different sources.

## CHARACTERISTICS OF TROPICAL AS OPPOSED TO TEMPERATE POLIOMYELITIS

### AGE INCIDENCE

Of recent years in America, Australia, and Europe there has been an apparent increase in the incidence of poliomyelitis attack

America, about 85 percent of cases were under 5, whereas now only about 50 percent are under this age This changed incidence has been attributed to improved social hygiene, which tends to lower the risks

of respiratory or intestinal diseases

the question is whether the disease is primarily one of infants.

in the tropics the incidence is higher among older children and adults

is immunized by previous exposure and subclinical infection, owing to the low standard of hygiene in infancy in these countries, the disease

is common only in the nonimmunized infant (Burnet, 1940, 1945, Fanconi and Zellweger, 1942)

been a number of outbreaks

(virgin soil) communities, where

been more uniform, with incidence

adults (International Committee, 1932) The diagnosis in some of

these cases is difficult. A recent epidemic in the tropics was among the 10 to 25 age

### RACIAL SUSCEPTIBILITY

It has been generally assumed that the incidence of poliomyelitis is higher in the tropics than in the temperate zone. This is based on faulty reporting, and I cannot believe that it implies a racial resistance. I can see no reason why the nervous system of the colored person is not just as

(2) There were a number of severe epidemics in British overseas territories, and these were thoroughly investigated by experts, I refer particularly to outbreaks in Malta, Mauritius, St Helena, and Singapore

temperate as well as tropical

#### CRITERIA FOR THE DIAGNOSIS OF POLIOMYELITIS

many other virus diseases, for example dengue and sandfly fevers, mumps herpes lymphogranuloma venereum and lymphocytic chorio meningitis as well as in bacterial infections. Abortive forms of equine, St Louis Japanese, and African encephalitis may give a similar clinical picture. It is therefore impossible to diagnose non paralytic poliomyelitis from clinical observation alone with any degree of certainty unless frank paralytic cases occur at the same time. Even so it is likely that some of the cases reported as nonparalytic

fever (McAlpine 1946) or of unidentified aetiology (Blackie and Blair, 1940) the so called Guillain Barré syndrome must also be remembered. It is doubtful whether milder attacks of polyneuritis

de with certainty from

late reporting make it evident that most figures published for the incidence of poliomyelitis must be inaccurate, and we should maintain a strictly critical attitude in the absence of laboratory confirmation of the clinical diagnosis. In particular, it is unwise to lay too great stress on reported differences in incidence in various localities.

The only reliable method of laboratory diagnosis is the isolation of virus from nervous tissue, nasopharyngeal washings, or stool by inocu



The majority of cases occur between July and October, and a few are recognized in winter or spring. It has been estimated that nonparalytic cases probably exceed paralytic by about tenfold. The distribution of cases in America is characteristically patchy in one year, with particularly heavy incidence in one or more localities. However, probably because of extensive road and rail travel, infection usually tends to be more widely distributed geographically than the case in Europe.

#### SOUTH AMERICA

Sporadic cases have been reported from practically every country and outbreaks from several. Few of the large cities, however, suffer the extensive epidemics of urban North America.

#### WEST INDIES

The disease is endemic in Barbadoes, Jamaica, Tobago, and Trinidad, but epidemics are rare.

#### GREAT BRITAIN

Until recently the usual picture has been of small localized outbreaks in the summer, with little tendency to wide dissemination, but in 1947 the disease reached epidemic prevalence, cases occurring in most parts of the country, the incidence was probably about 20 cases per 100,000, closely similar to that in America in 1946.

#### SCANDINAVIA

It was in Scandinavia in 1905 that poliomyelitis first appeared on the world scene as an epidemic, as distinct from a sporadic, infectious disease. Of more recent years there have been many severe outbreaks of epidemic proportions. Cases have also been reported in Greenland. Iceland has had some epidemics of particular severity.

#### CONTINENTAL EUROPE

#### THE MEDITERRANEAN AREA

The disease is endemic on the North African seaboard, in Algeria, Morocco, and Tunis.

In Egypt only a few cases and deaths per year are reported officially, yet an enquiry from paediatricians revealed that residual para-

susceptible as that of the white. Any difference in incidence is much more likely to be due to social habits and other factors. Thus in the Mauritius outbreak, an apparently greater incidence in Chinese children was probably due to the fact that their families were largely shopkeepers and were thus more frequently exposed to infection. Somewhat similar observations have been made in Hawaii (Lee 1941).

ter

Al

natives serving alongside, presumably because the native troops were immune to the local strains, whereas the visitors were susceptible.

It has also been alleged that a lesser tendency to develop paralytic as compared to nonparalytic illness is characteristic of tropical poliomyelitis. I very much question however, whether this is really so. When specially looked for children showing residual paralyses have quite commonly been found, e.g., in Egypt, Malta, and China.

#### ABSENCE OF LARGE SCALE EPIDEMICS

In most temperate climates, cases occur mainly in summer and autumn epidemics with a few sporadic cases all the year round.

In tropical lands, cases occur more evenly throughout the year, epidemics are rarely recorded but the total annual number of cases may be

Europe, but with the attack predominantly on the under fives. At present, epidemics in the tropics are being increasingly reported

### THE GEOGRAPHICAL INCIDENCE OF POLIOMYELITIS<sup>1</sup>

#### NORTH AMERICA

Epidemic poliomyelitis has been prevalent in the United States and

was approximately 20 per 100 000 inhabitants. In the past 10 years, there has been a considerable increase in epidemic prevalence in the Southern States, previously less severely involved.

<sup>1</sup>In the compilation of this section of the paper I have freely consulted the Monthly Epidemiological Reports of the League of Nations, e.g. for 1932, 1934, 1935, and of the World Health Organization for 1947, also the Report of the International Committee on Poliomyelitis (1937). Much useful information is also given by Simmons and his co-authors in the book *Global Epidemiology*. I shall not give extensive references in this paper as I have done so in more detailed reviews elsewhere (Rhodes 1947, van Rooyen and Rhodes 1943).



ralysis was not uncommon (Paul, Havens, and van Rooyen, 1944). The incidence of the disease appears to be more evenly spread over the year than is the case in North America, and epidemics have not been described. The main victims are children under 5, adult Egyptians being rarely attacked.

In Palestine the condition is said to occur mainly in Jewish children under the age of 5. Arabs are rarely attacked.

The disease is endemic on the island of Malta, but only 61 cases were notified from 1921 to 1941. From November 1942 to June 1943 there was a severe epidemic, involving 426 civilians and 57 service personnel. In August 1945 there was a small outbreak involving 10 cases.

At the end of 1945, again involving chiefly children under 5 years (Kauntze, 1946).

#### AFRICA

The infection is endemic in the Belgian Congo, French Equatorial Africa, and West Africa. Children are mainly attacked.

Small outbreaks have been described in North and South Rhodesia. Of recent years the disease has become increasingly prevalent in the Union of South Africa, and there have been a number of epidemics (Union Department of Public Health, 1945).

#### ST HELENA

This isolated island, which has a temperate climate, had a severe outbreak between November 1945 and January 1946. The majority of cases occurred in the 10 to 25 age group of the native population (Kauntze, 1946; Nissen, 1947). There were no less than 217 cases in a population of about 4,000, but no cases occurred in the garrison of European troops. The infection was probably imported by a carrier infected in South Africa, who spent only 2 hours on the island.

#### THE INDIAN OCEAN

The disease is endemic in the Indian Ocean.

#### CONTINENTAL ASIA

In India the disease is endemic.

As regards the question of race, which may interest people a good deal, I would like to mention some information which will confirm what Dr Rhodes has said, namely, about studies on the incidence of the disease in negroes and whites in the United States. I particularly would like to plot very quickly the reports of a survey reported by Collins of the United States Public Health Service, which showed that in the northeast of the United States the attack rate

in white and negro, which is low in both groups. The possibility that the lower incidence of the disease among negroes in the North and among whites and negroes in the South may be due to their poorer economic status is not borne out by the survey data which indicate that among whites in the same region the economic status does not affect the total amount of poliomyelitis.

I would like to bring up another interesting correlation. In the epidemic of 1930 in San Francisco, which has a considerable population

the attack rate was  
then the Chinese,  
We know that in  
but relatively rare

among Americans. I think all we can say, from a world tour is that, is that poliomyelitis is present wherever human beings are present but it does not mean that the consequences of infection with poliomyelitis virus are the same wherever human beings are present. One of the things that one is very much impressed with by studying the disease in different parts of the world, or even in different regions of one country, is that the paralytic consequences of the infection are

ion of the so called  
ult type of pattern

does not bear  
disease in a  
data kindly sent  
in London

is low and where epidemics are infrequent, probably there is more widespread dissemination of infection due to poor sanitation and hygiene, that holding for England as well as for certain tropical countries and especially for the congested communities of London. In accordance with this belief one might expect that when an epidemic started in such an area it would be infantile in pattern. Now, London

## CONCLUSIONS

Poliomyelitis has a world wide distribution, but there are differences between the biological characteristics of the disease in temperate and tropical countries. Epidemic prevalences in the tropics, previously rare, seem to be on the increase. There is urgent need for further

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#### ABSTRACT OF DISCUSSION OF PAPER BY RHODES

Dr ALBERT B SABIN (United States) Dr Rhodes has taken us on a world tour of poliomyelitis and I would like to comment merely on a few of the areas he mentioned in which I have had an opportunity to observe the manifestations of the disease

Campos of Sonora, Mexico He brought an excellent report of an epidemic that occurred in the adjoining Province of Sonora, this last January February and March 1931.

these first cases in the year occur in the southern United States and closely related countries and why do our epidemics farther north occur in the summer What are the factors which are responsible for these epidemiologic variations? We can't answer them at present, but we might suggest the possibility of new strains being introduced from the south, or consider the effect of climate on either the host or the virus as being responsible

Dr C G PANDIT (India) Perhaps this conference would like to hear of a recent epidemic we had in one of the islands off the Indian Coast. I refer to the islands known as the Andaman and the Nicobar groups of islands In October last year we received reports of an infection on the Nicobar Islands.

there in order to see whether the virus could be isolated The full report will be published in the Indian Medical Gazette.

were 800 cases with 400 deaths during a period of 2 months, and the age distribution was practically that all groups were affected, the majority being over the age of 10 The infection was evidently introduced from another island These islands are known as the

diseases This isolation island was a center for priests and every year these priests visited the other groups of islands Early in October, about one hundred people from the isolation island reached the particular island which I am referring to and 15 days later the epidemic arose Now in this island which was infected, the population lives

too, was infected, but no other investigations have been done in this second group of islands

All the equipment was transported by plane to this island and the epidemic was investigated There were no monkeys on that island so we had to transport monkeys from India We infected them with stools and just before I left two of my monkeys had come down with paralysis This is all that I can say at the present moment An

up until 1947, has had a very low attack rate, comparable to the lowest

high attack rate on individuals over 15 years of age that was seen in London and Berlin in 1947, and in Copenhagen in previous years. It would appear that there is no one pattern for the so-called countries of western civilization, they can be quite different. Age selection patterns which occurred in Sweden in 1905 did not appear in New York until 1931, and attack rates and patterns seen in Sweden now haven't as yet happened in Denmark or the United States.

It seems to me that neither the importation of new immunologic types of virus or of strains of especially high virulence can explain the occurrence of epidemics in some parts of the world and their absence in others. If new immunological types were responsible for epidemics, we should expect constantly and irregularly changing age selection patterns, which is not the case. If epidemics depended on

as especially virulent judging from the incidence and severity of the disease among Americans in those areas

There is I think, as yet no simple rule that we can apply to explain the peculiar patterns that are seen in various parts of the world

with the fact that cases first occur in the southern part of the United States each season and then work north. Several men in my department this year followed the weekly incidence reports through the year and noted that the first cases each year occur in the south, in Florida or in southern Texas, and then gradually work north. We have observed in Los Angeles each year that usually the southern part of the state, i. e., Imperial Valley or San Diego, is the first to be attacked and then the cases work north from that area. A very interesting observation was brought to my attention just a few weeks ago by Dr



## THE CUBAN RESERVOIR OF POLIOMYELITIS VIRUS

F. RAMIREZ CORREA, M. D., *Finlay Institute, Havana, Cuba*

Poliomyelitis, as an epidemic entity, was once characterized by its geographical limitations, but afterward, it had the special distinction of going along with man in his trends towards better conditions of living and hygiene through modern times. The frontiers of its domain have thus steadily broadened upon earth and its virus has found a permanent reservoir in every country.

Whether or not this reservoir presents particular characteristics and may or may not be an important part of the whole structure of poliomyelitis, is a problem which challenges today the skill of students of the disease. Countries located in the vicinity of the tropics are a good example of this concern. Through the last 25 years, epidemics have been described in Hawaii, Panama, Salva-  
Puerto Rico, and Cuba. The last epidemic in Cuba, 1946-47, was characterized by periodical visits, and some of the cases were described by F. Lopez.

We found it interesting to report some of the viral observations so far carried out in Havana during 1946-47 and to present some of the data related to the immunological evolution of the disease in this country, in order to give an approximate idea of the nature and extent of our reservoir.

### MATERIALS AND METHODS

*Data from the origin of poliomyelitis in Cuba*—The first epidemic of poliomyelitis in Cuba was officially described in 1909 (2), but the actual existence of persons with deformities compatible with the disease is prior to that date. To find out when the disease first came established in Cuba, attempts have been made to compile a birth census of its resultant deformities in people over 40 years old.

The data were collected by physicians who were practising prior to the Spanish American War, with that purpose. Whenever possible all persons with deformities acquired prior to 1909 were visited, and personal clinical histories of all illness related with such impairments were carefully recorded. In all instances the age of the subjects at the onset of the disease was too short for them to remember any personal details of their sickness, but fortunately in all but few instances one of their parents was alive and provided the required data.

*Materials from 1946 epidemic techniques and sources for viral isolations*—Viral isolations have been attempted from these different

other team is working there to evaluate muscle efficiency and to take steps to rehabilitate the population in their normal occupation which is that of collecting coconuts and climbing trees, and so forth. In view of the very considerable incidence and the large number of cases reported, we are particularly interested from the point of view of the strain and from the point of view of the possible spread to other groups of islands.

primary isolations of poliomyelitis virus. Daily temperature records were taken, beginning the day before inoculations were made. When more than one appeared with paralysis in a given group, usually one (1 year) and the fate of paralyzed aminations of cord and medulla

Sometimes the brain, occasion were examined histologically

—with each group of monkeys inoculated, several groups of at least 12 mice, 6 guinea pigs, and 4 rabbits were also run as controls, and in 2 instances, 2 groups of native Cuban rodents (*Oapromys pilorides*) were used in attempts for primary isolations. This animal has the external appearance of an enormous rat, and its weight attains sometimes 10 to 12 pounds. In one instance an attempt was made to transfer the virus from monkey cords to cotton rats (*Sigmodon hispidus*) and the mentioned Cuban native rodent (*Oapromys*)

### RESULTS

**Survey**—As shown in table 1, two completely unknown outbreaks have been detected by our survey. The first one occurred in Santiago

TABLE 1—Origin of poliomyelitis in Cuba, survey 1947 transition from endemic to epidemic period

Period	Year	Local outbreaks	Dominant age incidence	Original method of detection
Endemic	1903	Santiago	2	Interview of living persons with clear deformities (Census, 1907) Published report
		Isle de Pinos†	1	
	1906	Pinar del Rio	1	
		La Habana	(?)	
Epidemic	1909	Santa Clara	2	Do
	1934	La Habana	3	
	1942	Cuba	3	
	1946	Western Provinces	2	

† Probably virgin-soil epidemic in small island.

de Cuba 4 years after the end of the Spanish American war and 1 year after the evacuation of Cuba by the American Army (1903). Another outbreak, never reported before, was discovered by our survey. It

clinical history of 7 persons with actual deformities. The population was of some 1,000 inhabitants which actually makes a very high incidence rate, at least 7/1,000.

Other epidemics officially reported are: In 1909, reported by Lebredo and Recio (2), in 1934 by Ramirez Corria (3), in 1942 by Recio, Martinez Fortun y Cartaya (4), and in 1946 by Ramirez Corria and Fermoselle Bacardi (5).

human sources (1) spinal cords from fatal cases, (2) feces of pa-

tion. The remainder of samples was kept in buffered saline in the ice box or deepfreeze refrigerator. All the cords tested for primary inoculations were used within a month of storage in cold. Ten percent suspensions were prepared by grinding the fragments with glass powder in a mortar. The suspensions were centrifugalized at over 1,500 revolutions per minute. The supernatants were collected, and 1 cubic centimeter was injected into the brain of a rhesus monkey, and 20 cubic centimeters into the peritoneal cavity. Cultures were made in aerobic and anaerobic media and showed no growth or, at most, were contaminated with few saprophytic bacteria, but we never noticed meningitis or abscesses that could have been induced by such contaminants.

*Feces*.—Rectal washings with 300 cubic centimeters saline or distilled water were centrifugalized at low speed. The solid matter and the liquid phase were calculated to make 1 part of feces to 9 of liquid. Then the mixture was thoroughly homogenized in a blender, and then centrifugalized at 1,500 revolutions per minute. The supernatants

1. 2. 3. 4.

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was added in order to

nate was frozen again, and the next day, if no growth was observed, it was inoculated into the brain of rhesus monkeys in amounts of 0.5 cubic centimeter. A previous test showed that this procedure produced practically complete bacterial sterility of stools. In few instances, some rough coliform colonies were observed in the inoculum after 2 or 3 days of culture, but they were well tolerated by the animals. After being inoculated with feces collected from a patient with

with satisfactory results.

*Monkeys*.—The monkeys used for primary inoculations were always young rhesus weighing from 4 to 6 pounds. Once an animal was treated with suspected clinical material, it was never used again for

TABLE 3.—*Virus from feces of patients and carriers*

Samples	Technique	Results <sup>1</sup>
1 2 3 and 4	10 percent suspensions low speed centrifuge supernat 1 cc daily into each nostril of monkey 1 week	0/4
1 2 3 and 4	5 percent suspensions low speed centrifuge supernat 500 units penicillin per cubic centimeter 0.4 percent phenol for 6 hours icebox 0.5 cc intracerebrum	3/4
Pools A <sup>2</sup> Pools B <sup>3</sup>	Same technique do	1/3 0/3

<sup>1</sup> Numerators: number of monkeys paralyzed denominators number of monkeys inoculated. All positive with second passage.

<sup>2</sup> A: brother's patient

<sup>3</sup> B: brother's contacts

tion of the central nervous system, we used penicillin and phenol, as described in the sections on Materials and Methods. We have applied this technique to all contaminated material with satisfactory results.

**Comments.**—Epidemics of poliomyelitis have been shown to be present in Cuba since 1903, and there is a period between 1903 and 1909, in which the disease manifested its presence under the form of small successive outbreaks. Curiously, this is the period in Cuba characterized by the intensive sanitation works undertaken by the administration of Gen. Leonard Wood, whose program was immediately followed by the Cuban health authorities and resulted in the eradication of smallpox and yellow fever, our classic plagues.

The period of 25 years, between 1909 and 1934, was characterized by the absence of epidemics. Then the disease reappeared with an outbreak of some 500 cases and a high mortality rate in the city of Havana in 1934, and again in 1942 and 1946, as has been shown in table 1. The picture is that of two small epidemic blocks at both ends of a long period with no epidemic at all. We wonder if this fact, together with the observation that no significant change occurred in the age incidence rate (which remains around 2 years of age), is the expression of a long endemic stage rather than epidemic, and if, consequently, the Cuban people are actually much more exposed to the virus than the people of the United States and probably show a higher immunity level.

TABLE 3A.—*Differences between the 2 principal strains in invasive properties and recoveries through passages*

Strain	Incubation average (days)	Clinical course	Killed, protracted	Spontaneous death	Recoveries observations
I	5-7	Generalized paralysis prostration within 72 hours	9	2	Gross deformities <sup>1</sup>
II	9-17	Limited paralysis or only fever	0	1	17 plus 9 killed for passages mild atrophies.

<sup>1</sup> Observation of a second spontaneous attack 45 days after inoculation and 39 days after initial paralysis.

### SUMMARY

The actual characteristics of the Cuban reservoir of poliomyelitis virus, that is, the evolution of epidemics, and the observation of the virus in man are described.

The most surprising feature in this epidemiological survey is the long period that elapsed without new outbreaks between the two main epidemic periods, though it is well known that the disease existed in the so-called sporadic form during that time. The age incidence of the cases was under 2 in all the epidemics, and in one, more precisely, under 1.

**Viral isolations**—Eleven of 18 samples tested yielded positive results and 2 different strains of virus from human sources were maintained through passages in rhesus monkeys. No virus was recovered in any attempt to infect other laboratory animals, mice, guinea pigs, rabbits, or the native Cuban rodent *Capromys pilorides*.

As may be seen in table 2, six of seven fatal cases from whom spinal cords were removed yielded positive results. All but one died within

TABLE 2—Isolation of virus from spinal-cord material of fatal cases of Las Animas Hospital, La Habana

Number of cases	Days after onset	Results	Observations on virus passages
2	2	2/2 M	Extremely virulent strain causing prostration and fatal issue? from 1 of these cases
1	3	1/1 M 1/2 M	
3	4	1/1 M 1/2 M 1/2 M	Through passages animals recovered, most of them with deformities
1	21	0/1 M	Do
			Patient died in respirator after long period. Lesions in cord of regressive type

<sup>1</sup> Observation of spontaneous attack in 1 monkey prostrated and recovered with deformities second attack after 45 days from the initial inoculation

4 days. The negative case was a young adult receiving respirator treatment for 3 weeks. The histological picture of his cord showed an extensive necrosis.

recovered with gross deformities. In a group of seven of such apparently recovered animals, one developed a spontaneous second attack, 45 days after inoculation, and 39 days after the initiation of the paralytic period. The histological picture was typical of the acute phase. No passage was made with its cord.

Virus isolations from excreta of patients and carriers were also performed. Four of five samples yielded positive results. This is

in some of the  
 turned positive  
 To inoculate  
 the fecal material into the brain without producing bacterial infec-

ways of living on the incidence of poliomyelitis. It could reasonably

in which climate and race play such an important part.

### RECENT EPIDEMICS OF POLIOMYELITIS IN SOUTHERN AFRICA

Poliomyelitis has been endemic in Southern Africa for many years but epidemics were unknown before the First World War. Then early in 1918 the first extensive epidemic occurred. It seems that conditions brought about by war favor the spread of the disease, for the next extensive epidemic did not occur until toward the end of the Second World War, when in the summer of 1944-45 South Africa suffered a widespread epidemic. Soon afterwards Mauritius and St. Helena, islands on either side of Southern Africa, experienced their most severe epidemics. In the autumn of 1946, local epidemics occurred in Northern Rhodesia. In the summer of 1946-47 there were few cases in Southern Africa. Another epidemic, the worst yet, occurred in the recent summer of 1947-48. This was particularly severe in Johannesburg, South Africa's largest city, where, up to the end of April, nearly 700 cases had been reported.

### SEASONAL INCIDENCE OF EPIDEMICS

All these epidemics occurred during the summer and autumn. There were some notable differences in the peak incidence of cases. In the

temperate climate, the greatest number of cases occurred in mid December. In tropical Northern Rhodesia one outbreak occurred during September and October, the hot dry months prior to the rains. Another outbreak occurred in February and March in the midst of the rainy season.

In contrast with the 1944-45 epidemic, the recent epidemic of the summer of 1947-48 in Johannesburg began a month later, did not attain its peak until the middle of March, and maintained this peak through April. The reason for the seasonal incidence of poliomyelitis remains unknown.

In southern Africa there is a clearly defined rainy season and a clearly defined dry season. The rainy season is supposed to follow the September equinox. Sometimes it does. It did in 1944, when

reached its peak in mid December, then rapidly declined. This last

The significance of the low age incidence of the cases is emphasized because this probably indicates an extensive and constant contact with the virus, which results in a high immunity level of the adult population.

The results of viral isolations from spinal cords of fatal cases and from feces of patients and carriers are recorded. The finding of two strains of very different invasive properties is reported. Attempts to get some poliomyelitis rodent adapted strain from the isolated virus were negative.

#### ADDENDUM

In what concerns the possibility of dissemination of poliomyelitis virus by wild birds, there have been three ways of approach.

(1) Direct isolation of virus in feces of birds. In one of three attempts resulting were positive. However, the validity of this observation must be contested since the feces were collected on the soil in a public park and might have been contaminated by flies, or by human or animal carriage of the true virus.

(2) Another way of approach has been the observation that in wild birds experimentally fed with infected monkey cords, the virus is not destroyed in the oral tube, and, moreover, for the longest period tested in our short number of experiments (24 hours) remains fully virulent for new rhesus monkeys.

(3) The third way of approach has been the production of antibodies in four groups of birds injected or fed with the Lansing poliomyelitis strain, indicating that these animals react as if they had

question

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#### ABSTRACT OF DISCUSSION

Dr C. C. DAUER (United States). I would like to comment briefly on the two papers. I think that there is a factor that one must take into consideration when comparing the incidence of poliomyelitis



occurrence of widespread silent infections during the winter and other interepidemic periods. It naturally does not exclude the occurrence of a few cases.

Since January 1948 up to the present (April 1948), virus has been consistently demonstrated in the sewage. These regular examinations will be continued to determine when in relation to the end of the clinical epidemic the virus again disappears from the sewage. In looking for a source of virus to account for this seasonal incidence, a series of tests were carried out.

Batches of flies, including *Musca vicina*, *Lucilia sericata*, *Chrysomya* sp., and *Sarcophaga* sp., both adults and larvae, were trapped from the neighborhood of cases. The monkeys inoculated with suspensions prepared from these flies remained healthy. It is understood that the monkey used for these tests, *Cercopithecus aethiops pygerythrus*, is relatively insusceptible to fly virus, so these negative results do not exclude the possibility that poliomyelitis is flyborne. However, it is relevant to note that villages in Northern Rhodesia and Swaziland, which had been thoroughly and regularly treated with DDT, suffered their first epidemic outbreaks recently in spite of this treatment.

It has often been suspected that fruit and vegetables might be a source of virus. Suspensions prepared from fruit and vegetables (apples, figs, lettuce, and marrow) grown on a sewage farm and irrigated with the humus tank effluent were inoculated into monkeys. None of these monkeys developed typical poliomyelitis.

Migratory birds were also suspected because their arrival often coincides with the onset of the poliomyelitis season. Droppings from swallows hawking insects on a sewage farm were collected. After appropriate treatment suspensions prepared from these droppings were inoculated into monkeys. These remained healthy.

table 1

It is of interest to note that none of the wild caught vervet monkeys showed protection

TABLE 1.—Immunity tests with animal sera

Source	Number tested	Number immune	Percent immune
Animal sera			
Vervet monkeys			
Wild-caught	50	0	0
Post inoculation	20	1	5
Post paralysis	7	2	28
Rhesus monkeys	6	0	0
Rats	20	0	0
Bird sera			
Fowls	20	3	15
Ducks (post paralysis)	2	1	50
Pigeons	7	1	14

summer was different. There was no appreciable rain until early in December. Since then rain has fallen at regular intervals. Because

intriguing question

On comparing these two epidemic years, 1944 and 1947, it is

often been noted that there appears to be a relation between rainfall and the incidence of poliomyelitis. From our experience it seems unlikely that there is a direct relationship. However, there may be indirect ways in which the rainfall influences the incidence of polio

it is a subject that has often been investigated, it therefore seemed worth while to study again in southern Africa some factors which might influence the seasonal incidence of poliomyelitis.

It was necessary first to decide whether the infection was indeed seasonal, and not only apparently seasonal because of the occurrence of paralytic cases. It was considered that the presence of the virus of poliomyelitis in the sewage would reflect the infection of the community.

In January and February 1946, the presence of the virus of poliomyelitis was demonstrated in samples of settled sewage taken from a purification plant serving some of the suburbs of Johannesburg. The last occasion, February 19, was nearly 2 months after the last case of poliomyelitis had been notified in the area. It is thus clear that a silent epidemic was occurring at this time. Specimens collected in March and April of that year gave negative results. The collection of specimens was begun again in September 1946. Since then samples have been collected at monthly, and more recently at fortnightly intervals. After treatment with ether, 25 cubic centimeters of each sample of 500 cubic centimeters was inoculated into one vervet monkey. The sewage was not concentrated in any way prior to inoculation. The virus was not demonstrated in the sewage again until January 5, 1948. By this time, a fairly widespread epidemic was already in progress. The first cases of this epidemic occurred in the suburbs served by this sewage works in November. It is thus

fection is more widespread. If this is indeed so, silent infections must be frequent (table 2).

The occurrence of such silent infections in affected European families has been demonstrated on a number of occasions. Eight households in which there was one case of paralytic poliomyelitis were tested. In each case one or more other individuals were found to be infected. Most of these individuals had no complaints.

TABLE 2—*Immunity to poliomyelitis as shown by the Lansing strain mouse protection test*

Population	Age group	Number tested	Number immune	Percent immune
European	5-10	50	37	74
	Adults	50	40	80
African	5-10	40	35	88
	Adults	50	47	94

to our surprise three of nine monkeys inoculated, each with a suspension prepared from one specimen of faeces, developed typical paralytic poliomyelitis. Sections of their spinal cords showed the characteristic lesions.

The nearest paralytic case to this village was over 20 miles away. It is thus clear that a silent epidemic of an infection with the virus of poliomyelitis was occurring in this school at the time of the collection of these specimens. None of the children had symptoms suggestive of poliomyelitis. This finding emphasizes the extreme difficulty of the task of the health authorities in attempting to control the spread of this disease, in which unrecognized and unrecognizable infections may be common.

In this study confirmation has been obtained of the view that although paralytic cases are not so frequent infection is more com-

an immunity acquired and maintained by regular infections with endemic strains of the virus.

#### ABSTRACT OF DISCUSSION ON PAPERS BY RHODES, COERIA, AND GEAR

Dr W McD HAMMON (United States). From the standpoint of the epidemiologist, this problem of the apparent difference in the distribution of poliomyelitis in the tropical areas and in temperate

Of the 27 monkeys tested after inoculation, 7 had paralysis. Only 2 of these 7 gave a positive result. It is concluded, therefore, that during the epidemic a Lansing type strain of virus was responsible for some of the cases. Most of them, however, were due to a non-Lansing type of virus.

One monkey of 20, which were inoculated but did not become paralyzed, developed immunity to the Lansing strain. This monkey was inoculated with the faeces from a case of paralysis.

It is clear, then, that more than one strain of poliomyelitis virus is responsible for the present epidemic outbreak in South Africa.

At the time of the epidemic of human poliomyelitis in South Africa, there was also an epidemic of fowl paralysis affecting fowls, ducks, turkeys, pigeons, and canaries. On two occasions an emulsion of the spinal cord of a fowl dying of this disease was inoculated into a monkey. These monkeys remained healthy and did not develop protective antibodies.

In the case of one fowl, it was noted that its serum was protective before it developed paralysis.

At present, it is not possible to assess the significance of the protective antibodies demonstrated in the sera of fowls, ducks, and pigeons from flocks suffering from paralysis. Onderstepoort Veterinary Laboratories have undertaken an investigation into the aetiology of this epidemic of fowl paralysis.

#### RACIAL INCIDENCE OF POLIOMYELITIS

Previously it was noted that in southern Africa the European was more likely to contract paralytic poliomyelitis than the natives. This relative freedom of the African from the paralytic disease is of considerable significance.

Possibly there is a racial physiological factor concerned, the nature of which is not understood. It is well known that the African is much less liable than the European to certain other infections of the spinal cord and brain, such as tabes dorsalis.

The state of nutrition may be responsible, because the Bantu subsist largely on maize meal, whereas the majority of Europeans have a varied diet. It may be that their well nourished healthy tissues form a better medium for growth of virus than do the cells of the relatively not so well nourished Bantu.

The most likely explanation appears to be that the African in his more primitive surroundings was more regularly exposed to endemic strains of the virus and so acquired an immunity not shared to the same degree by the more hygienic European. In this immunity survey, it will be noted that in both sections of the population the majority are immune, but in each group tested the Africans have a higher percentage of immunes than have the Europeans. It thus appears that, although cases of the paralytic disease are rarer amongst Africans, in

I . . . . . I could find  
 . . . . . years, I

Also, I want to comment again on what Dr. Dauer said, that

age specific rates from San Diego and San Francisco, for example, we find that the age at which poliomyelitis is reported in San Diego is lower than that at which it is reported in San Francisco, and over a period of years the age appears to have risen in those communities. However, the difference is not nearly as striking when these rates are corrected for the population as when they are uncorrected. Therefore, again I would like to urge that we hesitate and wait in the interpretation of these results.

that hasn't been reported. I spent 4 years in the Belgian Congo, in the tropical medicine in Brussels in which not only the importance of the disease is represented but also the importance of the disease is represented within the community. I ran competition only with the white diseases. I couldn't help frequently went to the white diseases.

adult who was paralyzed in such a way that he might have had poliomyelitis.

In Central America a few years ago in one of the smaller countries I was asking particularly about poliomyelitis and no one could show me a case at the moment, although they assured me that occasionally they had it. But at a clinic we had a case presented to us as an example of cerebral malaria which appeared to me to be typical postparalytic poliomyelitis—malaria organisms had been demonstrated in the blood. So, though we frequently hear of cases in the tropics, the diagnosis, and the results are a little.

little. I saw no measles, no chickenpox, no mumps—though the diseases were reported before and afterward—so poliomyelitis might have been present also.

Dr. JOHN F. KESSEL (United States). I have a question with reference

areas is rather fascinating As you have heard from the papers this

where there is poor sanitation than in temperate areas where sanitation has been improved, that the disease is more frequently manifest

S

fancy and the very young age group I think it is an interesting hypothesis and quite a bit of evidence has been brought forth this morning in confirmation of this Yet, I think we need to be extremely cautious before going overboard There are so many questions yet that cannot be answered

All these papers this morning have also suggested we should be cautious in the interpretation of the data from the neutralization tests done with the Lansing strain of virus in the mouse We don't know what the results mean We as yet don't know how specific a test that is for indicating infection with poliomyelitis virus

I call to your attention some data I have already published indicating the widespread finding of antibodies against this virus in animals, somewhat parallel to those Dr Gear presented We found large numbers of chickens and wild birds with antibodies Yet when we inoculated the birds in the laboratories antibodies did not result from

antibodies in different human age groups require careful interpretation For instance, such antibodies may be present in one community over a period of 1 year as a result of a great deal of inapparent infection and then perhaps they may be relatively absent from the community for several years Thus if we take a survey in 1946 of one area, and compare it with another area in 1946, we might find more antibodies in one area than in the other Perhaps there would be differences in two racial groups adjacently located I am sure if we did such a survey with influenza antibodies, we would find that

Now, if I made the same survey three or four years from now, I am

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Dr I RAMIREZ CORREA (Cuba) If I interpret the question Dr Hammon correctly as to the distribution of cases in the tropics, he was in Colombia particularly in the last 20 years, every 3 or 4 years they have had such outbreaks all over the country in Colombia and if I remember correctly, I think the same thing has been observed in Venezuela, too

Dr F O MACCALLUM (United Kingdom) It is with some diffidence that I arise in such a gathering to tell what I have seen

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 investigation of this subject. We had commenced, for one to commence an investigation to collect sera throughout the country, but unfortunately very few sera had been collected in the different age groups when we went off to Scandinavia in July for the Microbiological Congress, and while I was there I discussed this problem with the authorities, with Dr Paul and Dr Sabin. We had always wondered why we didn't have poliomyelitis in England and what we would do to study this. But unfortunately fate was moving somewhat faster than we were and while we were sitting there having dinner and discussing this, the epidemic was rising in England. I don't think any of us know yet why this epidemic occurred. It was the greatest that has ever been recorded in England—figures should be mentioned to you but I question how accurate they are

With regard to the epidemic in England, the Ministry of Health in England, the Poliomyelitis Congress it except it was interesting. But what many of the concentrations of cases occurred in exactly the same places as they had occurred in the epidemics in the early part of the century. The disease appeared to occur more or less simultaneously throughout the country. There were earlier concentrations and later on there might have been some spread, but it wasn't very convincing

ence to the presence of the neutralizing antibodies in fowls, birds of one sort or another, chickens and so forth. I followed the work of Dr Hammon in this connection with interest in California, and we in the southern part of the State have done some work on this same subject with corroboratory results. I was much interested in Dr Gear's report this morning. My question is, has anyone studied the possible relationships of the Lansing strain of poliomyelitis virus to the Newcastle virus?

Dr A. B. SARRIN (United States). I believe that a few words of caution are needed about the use of the Lansing strain of poliomyelitis virus in studies on the epidemiology of the disease. There are great errors inherent in tests with this virus when small doses are used for neutralization tests. Dr Hammon speaks of fowl having antibody for Lansing virus before infection and losing it after they are inoculated with the virus. I think one can reproduce the same thing with animals that have not been infected merely because the results with small doses of virus can be very irregular. In my laboratory extensive tests have been carried out with the Lansing virus. We find that when small doses of virus are not used one cannot demonstrate antibodies in animals and fowl comparable to those found in human sera. We have also tested animals and fowl from Okinawa

tropical areas, far eastern areas, and the United States, and a study

data on the incidence of poliomyelitis in Japan, 1932-1935.

crucial. As Dr Ramirez Corria has pointed out, in most of the epidemics with an infantile age selection pattern the incidence is highest in 1 and 2 year old children. We have to determine, therefore, whether in regions without epidemics those 1 and 2 year-old children develop their own antibodies so fast before the complete disappearance of their maternal antibodies, that they do not get paralytic poliomyelitis when they are exposed to the virus in later years. Thus we found that in Cincinnati (which may be taken as a representative American city) the antibodies begin to reappear slowly at about 9 months of age, but the process is relatively slow so that by the time 5 years of age is reached only 40 percent or less are positive. On the other hand similar tests on sera of children from Okinawa, Korea,



question as to the age distribution of poliomyelitis cases in tropical countries and in countries where the disease is endemic, as opposed to countries in temperate climate where it is epidemic, and where we have been accustomed to see it appear in older and older children. This is the point that seems as a valuable new aspect if properly interpreted, nations become immune

Perhaps it is worth while to reiterate, as Dr Dauer has emphasized, that our data are probably incorrect and that we should not be glib in reporting age percentages of cases in various series in various parts of the world. Age specific rates are necessary if we are going to get any real knowledge of what is going on in age distribution, and I do not see how anyone could quarrel with that.

Dr Sabin, if I interpreted him correctly, feels we should be ultra cautious in trying to tie this thing up with obvious immunity processes that might be going on in tropical countries where there is a very wide distribution of virus, where children and infants may be exposed to

infection

A third aspect of this same discussion is whether or not we can link up the past history of poliomyelitis, within a given community, with this immunity of the inhabitants and with the age distribution of the disease in that community. And there I think the observations of Dr Gear come in. How many silent epidemics do we have, how often does the disease exist as an 'intestinal infection' never penetrating as far as the central nervous system? We know very little about the history of poliomyelitis in many communities. Certainly the official records give us a very inadequate story of how much virus distribution and how much immunizing infection has actually taken place.

I, too, would like to emphasize the fact that we should be cautious in interpreting these interesting new aspects of the disease. But I

I shall speak of the occurrence of poliomyelitis in the true tropics of Africa. During the war we had epidemics, or local outbreaks, rather, of poliomyelitis in Northern Rhodesia, an area which is well within the tropics and has an average maximum temperature for most of the year of over 90. It was interesting that more or less at the same time and in the preceding year there were outbreaks in the Belgian Congo. All these

.. .. Africa to the thought the these areas

So we took material from fatal cases in order to have a histologic confirmation, rather than stools. I had only 8 or 10 monkeys, and we took material from 8 different cases from 8 separated parts of the country, Ireland, Scotland, Wales, and the southern part of England and the Midlands, and we isolated 4 strains from 4 different cases, 1 from northern Ireland, 1 from Scotland, 1 from Wales and 1 from London. The incubation period was exactly the same and the disease in the monkeys was approximately the same, with some variation, of course, in severity. We then tried to adapt the virus to rodents, but we have been unsuccessful both in the rat and

time, we had a vast increase in the recorded cases of neurotropic

infectious by inoculating a monkey at the same time, and tried to pass this through the dogs, carrying it through three blind pas

as many speakers have said, what this means, but we feel if we can produce a picture of this for England

months and perhaps that will be of some use to those who are studying this problem throughout the world

Dr ROY F. FLEMSTER (United States) Dr Gear injected into his talk the problem of cerebral syphilis and brought up the query in my mind as to whether there is any difference in the life spans of the African, the European, and the South African. I wonder if he has any data on that.

Dr JOHN R. PEARL (United States) It has been of considerable interest to me to note how the discussion has shaped itself around the

## Session 6. ARTHROPOD BORNE ENCEPHALITIDES AND RABIES

*Monday, May 17—9:30 a m to 12:00 m*  
*Department of Commerce Auditorium*

### JAPANESE B ENCEPHALITIS<sup>1</sup>

W. McD HAMMON, M D, DR P. H

It is not my purpose either to duplicate or to enlarge upon the several partial reviews of the literature of Japanese B encephalitis which have been written in recent years (1-4). More than 100 articles on this subject have been published by Japanese physicians and scientists alone<sup>2</sup>. In this paper, an attempt is made to discuss a few of the previously known facts about Japanese B encephalitis and some of the newer knowledge which relates principally to the potential importance of this disease in areas of the world outside Japan<sup>3</sup>. An effort is made to cover most completely those aspects related to its tropical distribution.

Japanese B encephalitis is a very highly fatal, explosively epidemic disease, caused by a filterable virus. Until World War II, it had been generally considered to occur almost exclusively in Japan itself, and there was only limited interest in this disease in the United States, and even less in some other countries.

It was not until the United States became engaged in war with

Japan that the Japanese used To develop means to protect troops in combat and occupation forces while they were in areas where the disease had been previously reported and epidemics might be expected to occur; (2) to devise means

<sup>1</sup> Original work was carried out in collaboration with the Commission on Virus and Rickettsial Diseases, Army Epidemiological Board, Office of The Surgeon General, U S

#### IV VIRUS AND RICKETTSIAL DISEASES

and were probably virus strains of different immunological genericity or more virulent than the endemic strains

Regarding the age incidence, I think our figures, particularly these mining villages, are accurate. I might add, although it is in my paper, that we have carried out surveys in Northern Rhodesia

of the European, but there are many, many thousands of them, many of them possibly, who live into the age group where one does get manifestations of syphilis, such as *tabes dorsalis* and general paralysis



to prevent its accidental introduction into Pacific islands under our control or into the western United States, and (3) to perfect methods by which to recognize and control it, should the virus be introduced either accidentally or intentionally. Introduction into the United States by intention was a matter of serious concern at the time when Japanese balloons began floating in from over the Pacific.

For the most part, these wartime investigations have now been reported. A formalin inactivated vaccine, first of mouse brain (5) and later of chick embryo (6, 7), was developed and put into use (8-12). Advances were made in the techniques and interpretation of serological tests for more rapid and accurate diagnosis, including the development of complement fixing antigens (8, 13-20). The possibilities of vectors and of hosts and reservoirs other than man were also explored (15). Much of this preparation for the protection of troops found direct application in Okinawa during the summer of 1945, just at the end of the war, when our Army and Navy units found themselves in the midst of a sharp epidemic of encephalitis among Okinawan natives. At least 12 severe cases, with 2 deaths,

by neutralization tests, and by isolation and identification of the virus agent (8, 22). Vaccine of the mouse-brain type was flown to the island and the new product given a mass field trial, unfortunately too late to give any evidence of its effectiveness, though its harmlessness was established (8). Since, among American epidemiologists, opinion on the mode of transmission was in favor of mosquitoes, extensive mosquito control operations were put into effect as quickly as possible, and before vaccine became available.

feeling that the characteristic explosiveness of known epidemics rendered any vaccination program begun after the recognition of the disease of doubtful value. Subsequent experience has tended to confirm this opinion. In addition, mosquito-control measures were recommended for both Okinawa and Japan. The Army's consultants recommended also that Korea be regarded as a place of potential danger. These recommendations were acted upon. British forces in Japan were also supplied with vaccine and were given all available information regarding the potential dangers of the disease. However, it was among nonvaccinated American occupation troops in Korea that the disease actually struck in the summer of 1946 (21).

the findings of the two groups of workers hinge upon one essential variation in technique. All workers, including Mitamura and his group, were usually unable to demonstrate virus in field caught mosquitoes and in the blood of patients by direct inoculation of mice. Mitamura and Kitaoka, however, found that "natural virus had to be 'adapted' to this new host by a series of "blind passages" usually three to six. It then became highly pathogenic for mice. The other workers either regarded this technique as superfluous or avoided it.

In our laboratory in California during the war, we were readily able to effect transmission to mice of the Japanese B virus by seven local species of mosquitoes from the genera *Culex*, *Aedes* and *Culiseta* (29), but in a small number of tests we were unable to demonstrate congenital transmission. However, largely on the basis of the very convincing evidence for mosquito transmission of the St. Louis, Western Eastern, and Venezuelan equine viruses, and the many apparent epidemiological similarities observed between all members of the group, we have believed in the mosquito transmission hypothesis for Japanese B virus (2).

Because of certain similarities in appearance between *Culex tritaeniorhynchus* (strongly suspected by Mitamura, Kitaoka et al.) and *C. tarsalis*, our important vector of the encephalitides in the western part of the United States (30), we were particularly interested in studying the habits of *C. tritaeniorhynchus* in Japan and Okinawa.

#### *C. pipiens*

Thus we confirmed part of the work of Mitamura and his associates.

Although during the past three summers many thousands of mosquitoes have been collected from Japan and Okinawa by several groups of American workers to date no report of the isolation of a virus emanating from these collections has been published. Our results from mosquitoes collected in Okinawa during 1945 were negative (15), but tests on those collected from Japan and Okinawa in 1947 and from Guam in 1948 are not yet complete. Most of these mos-

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China has been amply confirmed by Americans (24, 26) and others

identified as active in all the main islands of Japan, on Okinawa and Formosa, and now on Guam. Antibodies to the Japanese B virus have also been reported from several areas in Africa (28). Thus it appears that this virus, or others closely related to it, is widespread in the eastern and western hemispheres in temperate and in tropical areas. It may still be found in many other countries either as a newly introduced epidemic agent or suddenly, though previously latent, in epidemic form. It is therefore of considerable importance to uncover the epidemiological behavior of this virus, so we may be prepared to cope with the disease wherever it appears.

In Japan two conflicting concepts of the means of transmission of Japanese B encephalitis are held. The one most commonly accepted and which was considered as official, at least until the influence of American occupation, is that of contact transmission, chiefly by means of inapparent carriers. The other school of thought, championed principally by Drs. Mitamura and Kitaoka, is that of mosquito trans-

failed consistently in their attempts to confirm most of the experiments with mosquitoes. On the other hand, Mitamura, Kitaoka and their associates have reported that they have repeatedly isolated virus from mosquitoes caught in epidemic areas, infected other mosquitoes in the laboratory, transmitted the infection to laboratory animals by

of naturally infected patients, dogs, mice, and other animals. *Culex*

the quantity of virus was titrated from various dissected portions of infected mosquitoes during and after the extrinsic incubation period. The virus was reported as eventually having attained extremely high titers in the salivary glands. Most other workers in Japan failed to find virus in field-caught mosquitoes and in the blood of man, and also failed to effect transmission by mosquitoes in the laboratory except when the mosquito



species of mammals might have virus circulating in high titer at some time during the period of infection, and that they could thus serve as a source of infection for mosquitoes. However, inasmuch as Okinawa was so depleted of these animals during the war and since, it would appear that on that island if any large mammal served as a source of infection for mosquitoes, man alone was present in adequate numbers to have served that purpose.

We have little data as yet with regard to the actual infection rate

in Guam,

Actual

all areas

However, whether virus circulates in the blood of an infected man in

arily becomes infected. Furthermore, the effectiveness of our vaccine has yet to be demonstrated. However, insofar as transmission during

1st We therefore invite further attention to this disease by members of this Congress and hope that we have stimulated your interest

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(34) also found the Japanese virus in the blood of inoculated chickens and detected its presence in that of ducks as well but their tests were not of a quantitative type. More recently we have obtained more encouraging results with experimentally inoculated house finches. Following a minute inoculation, virus was detected in very large amounts in the blood of several of these (15). Such titers of Western equine and St. Louis virus have proved to be entirely adequate for mosquito infection.

Thomas and Peck reported positive tests for virus from the blood of one inoculated horse (35). Meiklejohn and associates also reported positive results from three pigs, one of four goats, and one of four horses (34). All these were inoculated intravenously with from 1 to 2 cubic centimeters of a 1 or a 10 percent infected mouse brain suspension of Japanese B virus. Since the amount of virus inoculated was so much in excess of that which a mosquito might be expected to inject, little significance can be placed in these results. These experiments with large mammals should certainly be repeated with more appropriate doses of virus.

In our studies on Western equine and St. Louis encephalitis, a survey

#### source of virus

In such surveys for Japanese B virus infections Sabin reported that the sera from chickens in Japan and Okinawa were free from neutraliz-

epidemic on Okinawa in 1945, there were very few large wild or domestic birds present, but there were large numbers of small swallows. These were not tested for serum antibodies. Tests for neutralizing antibody made by us on 15 crows were all negative but 1 of 2 thrushes tested was positive (15).

In all surveys made a high proportion of the large domestic mammals in epidemic areas has been shown to have neutralizing antibodies, suggesting previous infection. Tests have been made on horses, cows, goats, and pigs (8, 15, 21, 37, 38). Our results on Okinawa in 1945 not previously reported were 7 of 10 cows positive, 8 of 9 pigs, 4 of 8 goats, and 27 of 30 horses. It is quite possible that 1 or more of these

# NEUROTROPIC VIRUSES IN CENTRAL AFRICA<sup>1</sup>

K. C. SMITHBURN, M. D., *International Health Division  
Rockefeller Foundation*

A dissertation of this brevity on a subject of such scope requires at the onset a definition of the term neurotropic. As here used, the term designates those viruses, the principal focus of attack of which is the central nervous system, and the lesions induced by which are localized principally or wholly within that system, whether or not the attack and the resultant lesions primarily involve nerve cells.

A considerable number of the viruses which are known to be capable of provoking disturbances in the central nervous system of man and/or animals occur and are active from time to time in Central Africa. Being engaged primarily in investigations on yellow fever, our first and foremost concern has been the virus of that disease, an agent which has a definite affinity for nerve cells although that affinity is, under normal circumstances, completely overshadowed and masked by its hepatotropism. Six times we have, by accident, encountered Rift Valley fever virus (1), another normally hepatotropic agent having thinly veiled neurotropic potentialities which may be demonstrated, even enhanced, by experimental methods. Immunological evidence has been obtained of the occurrence in Africa of infections with St

speak, by accident, in the course of investigations on yellow fever. Investigations of the new viruses have not been exhaustive in any instance. Nevertheless, the ground work for further studies has been laid, and the results obtained thus far will be discussed briefly.

## BWAMBA FEVER VIRUS

In 1937 Mahaffy encountered an epidemic of a fairly well defined clinical entity and isolated from the blood of nine of the patients strains of virus which proved to be identical with each other, yet different from other known viruses (3). The disease was called Bwamba fever in respect of the county in western Uganda where it was encountered.

# IV VIRUS AND RICKETTSIAL DISEASES

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variety of routes of inoculation than Bwamba fever virus. It causes encephalitis in monkeys when introduced intracerebrally or intranasally, and in hamsters by neural or extraneural inoculation (8). The lesions caused by it are characterized principally by necrosis of nerve cells without the formation of inclusions but are not readily distinguishable from the lesions induced by certain other viruses. Although West Nile virus is more common than the St. Louis virus, it has not yet been compared

### SEMLIKI FOREST VIRUS

This virus was isolated (9) in 1942 from a lot of 130 *Aedes (Aedimorphus) abnormalis* Theobald group mosquitoes caught in a relict strip of primary forest then continuous with the main Semliki Forest in western Uganda. It has never been isolated again from any other source, yet immunological tests (10) indicate that it has been active in both human beings and wild primates over a large area in Uganda.

Semliki Forest virus has been successfully cultivated in developing chick embryos (11) and its rate of growth in them determined. The virus reaches its maximum concentration in the embryos in about 16 hours and causes death of all embryos within 24 hours of inoculation.

The agent is not labile under ordinary conditions of laboratory procedure. It is more resistant to heat than most mammalian viruses.

lower concentration) and therefore also in all the viscera. The kidneys show a greater concentration of the virus than can be accounted for by the presence of the agent in the blood.

The lesions induced by Semliki Forest virus are limited to the central nervous system, with exception of the kidneys. The latter usually show hyperemia especially of the glomeruli, often to the point of obliteration of the capsular spaces. Hemorrhages in the kidneys are occasionally seen. There is usually interstitial mononuclear cell infiltration in the medullary portion of the kidneys. It is not possible to state whether these lesions are caused directly by the virus but they have been seen in every animal examined.

The lesions in the central nervous system are characterized by necrosis of the neurons and by extensive infiltration of polymorphonuclears but quantitatively highly variable. Hemorrhages are occasionally present and often the only feature not unlike those induced by the equine encephalomyelitis viruses (8).

#### IV VIRUS AND PICKETTSIAL DISEASES

Brimba fever is an

only noteworthy sign of the fever. Convalescence is rapid and complete. The moderate conjunctival injection and the appearance of the tongue. The latter shows moderate furring, with bright red margins and tip. The disease is noneruptive, so far as is known, but all the observed patients were Africans, and an inconspicuous rash could have been missed.

Blood was taken from the patients when they were first seen. If physical examination or blood smears revealed no other cause of the illness, mice were inoculated intracerebrally with the serum. Nine of a much larger number of patients with similar symptoms yielded strains of the virus. Inactivated acute-phase sera of these patients contained no neutralizing antibody, whereas convalescent sera of each neutralized the first strain of virus isolated. Intracerebral neutralization tests with antisera against a number of other viruses have demonstrated no cross neutralization.

Brimba fever virus is one of the largest of the neurotropic viruses. Ultrafiltration experiments indicate that it has a particle size of 113m $\mu$  to 150m $\mu$ . The agent is neurotropic in the sense that, in experimental animals, it attacks certain nerve cells (notably in Ammon's horn) with resultant formation of intranuclear acidophilic inclusions and also in that it is pathogenic for adult experimental animals only by intracerebral or intranasal inoculation.

Certain properties of the virus will be brought out in the general discussion which follows.

#### WEST NILE VIRUS

In 1917 Burke, working in the West Nile District of Uganda where yellow fever was known to have occurred, was searching for that disease among persons who appeared for routine sleeping sickness inspection. From a woman who had a mild fever without other obvious use or noteworthy physical signs he took a specimen of blood, the sum of which was inoculated intracerebrally into mice. This resulted in the isolation (1) of a virus which was named West Nile virus and which later proved to be related immunologically to the viruses he found in St. Louis and Japanese B encephalitis (5-7). No more than is known about the clinical nature of effects of West Nile virus in humans over a wide area in Central Africa, but this agent has been found to be effective in greater dilution and by a greater

variety of routes of inoculation than Bwamba fever virus. It causes encephalitis in monkeys when introduced intracerebrally or intranasally, and in hamsters by neural or extraneural inoculation (8). The lesions caused by it are characterized principally by necrosis of nerve cells without the formation of inclusions but are not readily distinguishable from the lesions induced by certain other viruses. Although West Nile virus is immunologically related to the viruses of the St. Louis and Japanese B encephalitides and possibly also to that of louping ill (5) it is not identical with any virus with which it has yet been compared.

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in the brains of infected mice but also in the blood (albeit in much lower concentration) and therefore also in all the viscera. The kidneys show a greater concentration of the virus than can be accounted for by the presence of the agent in the blood.

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to state whether these lesions are caused directly by the virus but they have been seen in every animal examined.

The lesions in the central nervous system are characterized by necrosis of nerve cells usually singly or in small groups, and tiny foci of infiltration around the necrotic cells. Pyknotic degeneration of Purkinje cells also occurs in some, but not all animals, with associated infiltration of polymorphonuclears. Perivascular cuffing is constant but quantitatively highly variable. Hyperemia of the brain is a constant feature and often the only macroscopic finding. Microscopic hemorrhages are occasionally present. The lesions in the brain are not unlike those induced by the equine encephalomyelitis viruses (8).

#### IV VIRUS AND BICKETTSIAL DISEASES

##### BUNYAMWERA VIRUS

Another apparently hitherto unknown virus was isolated (1943 from a large lot of *Aedes* mosquitoes caught in the Semhiki Forest in an uninhabited locality known to Pygmy hunters as Bunyamwera because of which the agent has been called Bunyamwera virus. A lot of mosquitoes comprised 14 species belonging to 5 subgenera it is quite impossible to state which was involved.

During the mosquito catch which yielded the virus, two color monkeys were shot in the forest area where the catch was in progress. One of these was subsequently found to be immune to the virus. Neutralizing antibody was also found in the convalescent serum of a man who suffered an attack of febrile illness characterized by marked neurological signs, and in a number of persons sampled at random. Immunological tests have also shown that the virus is unrelated to each of the other known viruses with which it has thus far been compared.

In its pathogenic properties for mice Bunyamwera virus is not unlike Semhiki Forest virus, yet it induces a different clinical reaction in them and is never effective in such great dilution. Mice receiving Bunyamwera virus exhibit marked hyperactivity and excitability and often die during convulsions in this state of excitation. The agent exhibited marked and unusual adaptative changes during serial passage in mice. It is pathogenic for and lethal to guinea pigs by intracerebral, intranasal or subcutaneous inoculation. It induces fever but no other obvious signs in rhesus monkeys when inoculated intracerebrally or subcutaneously. Rabbits are refractory to its pathogenic action, but they develop neutralizing antibody following inoculation. Lesions induced in the brains of mice are unexpectedly inconspicuous in view of the striking symptoms induced by the virus in these animals. Vesicular or pyknotic degeneration of nerve cells occurs but is diffuse in character and associated with little or no leucocytic reaction. Hyperemia is marked, and hemorrhages in the brain sometimes occur. Inclusion bodies have not been found.

The only lesions observed outside the nervous system are congestion and occasional hemorrhages in the kidneys, with moderate degenerative changes in the renal tubular epithelium and albuminous deposits in the lumina of the tubules. Whether these renal lesions are primary effects of the virus is not known.

##### MENGO ENCEPHALOMYELITIS VIRUS

A poliomyelitis like virus was isolated by my associate, Dr G W Aick (13), in 1946 from the spinal cord of a paralysed rhesus monkey which had been subjected to no experimental procedures. A second animal of the same virus was isolated from the blood of another rhesus monkey which exhibited fever when brought to the laboratory from outdoor runs. In the interval between these two isolations from rhesus monkeys, two strains of the virus were isolated (13) from



*Taeniorhynchus* mosquitoes (one lot of which was caught in and around the monkey runs), one strain from a wild mongoose trapped in the Institute compound, and one strain from the blood of a human

are characterized (15) by necrosis of the motor cells of the anterior horns and by perivascular infiltration. In some animals necrosis of nerve cells in the brain occurs also. The lesions are similar to those of poliomyelitis.

The one case of illness known to have been caused by this virus in man (14) may have been a laboratory infection. On the other hand the victim resided not more than 100 yards from the open air monkey runs in which two observed cases occurred in monkeys and several other animals became immune, and around which mosquitoes were caught from which the virus was isolated. He therefore may have acquired the infection naturally. His illness was characterized by intractable headache, fever, delirium, stupor, mild, transient palsies and by nerve deafness in one ear, which is the only sequel. The illness lasted about 10 days but left the victim in a weakened condition, recovery from which required about 3 weeks.

Mengo encephalomyelitis virus is highly pathogenic for mice guinea

viruses with which it has been compared

#### COMPARISON OF THE FIVE VIRUSES

The following tables show the origins of the five viruses and the species in which immunity to them has been found (table 1), their comparative virulence for mice (table 2), and a schematic presentation of their relative virulence for the more common laboratory animals (table 3).

In table 1 it may be seen that immunity to each of the viruses has been found in man. The studies are variable in scope, so that no attempt can yet be made to evaluate the relative incidence of infection with each. Nevertheless, it is known that the incidence of immunity to Bwamba fever or West Nile virus is very high in some localities and that immunity to Semliki Forest virus is commonly encountered in man. The studies on the other two viruses are much less comprehensive, yet they have shown that humans acquire immunity to the

## BUNYAMWERA VIRUS

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# IV VIRUS AND RICKETTSIAL DISEASES

Each of the human or animal sera tested against these viruses has also been tested against at least one other virus (yellow fever). No correlation has been found between the occurrence of immunity to yellow fever and immunity to any of the other viruses.

TABLE 1—Sources of viruses, occurrence of immunity against them in man and animals and their thermal death points

Virus	Isolations from—				Humoral immunity found in—			Inactivated by heating 30 minutes at 56° C.
	Man	Monkeys	Other animals	Mosquitoes	Man	Wild primates	Other animals	
Brantha fever	2	0	0	0	+	+	+	60
West Nile	1	0	0	0	+	+	+	45
Semi ki Forest	0	0	0	0	+	+	+	62
Bunyamwera	0	0	0	1	+	+	+	68
Mengo encephalomyelitis	1	2	1	2	0 <sup>1</sup> (18)	(20) 0 <sup>2</sup>	+	>80

<sup>1</sup> Very high incidence in some localities  
<sup>2</sup> Indicates no tests made  
<sup>3</sup> Figures in parentheses indicate number of animals tested  
<sup>4</sup> When suspended in 10 percent of non immune serum in physiological saline

Table 1 also shows that four of the viruses apparently attack wild primates, as monkeys have repeatedly been found to have neutralizing antibody against 1 or another of them. Only 18 sera of wild African primates have thus far been tested against Mengo encephalomyelitis virus, but none of these contained antibody. Absence of immunity could, of course, occur if the disease were invariably fatal in that species in nature.

TABLE 2—Comparative virulence for adult mice

Virus	Intracerebrally			Relative virulence <sup>1</sup>			
	MDO	AST	Titre	I-C	I-N	I-P	S-C
Brantha fever	4.8	4.8	64,000	++	++	++	++
West Nile	2.8	4.2	47,000,000	++	++	++	++
Semi ki Forest	1+	1.6	694,000,000	++	++	++	++
Bunyamwera	1+	2.0	5,000,000	++	++	++	++
Mengo encephalomyelitis	1+	1.8	22,000,000 LP <sup>2</sup> (284,000,000 LP <sup>3</sup> )	++	++	++	++

MDO and AST indicate mean day of onset and average survival time respectively after inoculation of 10 percent mouse-brain virus.  
<sup>1</sup> I-N, I-P, and S-C indicate, respectively, intranasal, intraperitoneal, and subcutaneous inoculations.  
<sup>2</sup> And LP indicate low and high passages.

Table 2 illustrates the variations in mean day of onset and survival in mice inoculated with 1 or 10 percent suspensions of each of the viruses. Exact figures for mean day of onset cannot be given for Semki Forest, Bunyamwera, and Mengo viruses as, with only once inoculations and death occurring soon after the first objective signs, the mean day of onset and the average survival time do not differ significantly. The data for each virus are derived from 68 to 93

## ABSTRACT OF DISCUSSION

were primarily interested in the study of yellow fever. It is worth pointing out that these viruses, and some others which he has described, turned up purely incidentally to the yellow fever work. The field studies in which they were isolated were primarily yellow fever studies, and the methods used were those known to be successful in the isolation of yellow fever virus. Bearing that in mind, I think, gives some indication, as Dr. Smithburn has said, of the possibilities for virus research in Africa.

Dr. A. B. SHERMAN has given us the main points in the 10 years in Africa, has uncovered a multitude not only of new viruses but new ideas for our consideration. I hope that what I am to bring up will not be interpreted in any way as critical of what has been presented. I merely want to present thoughts which come to those of us who wonder how to interpret the importance of neurotropic viruses, isolated particularly in mice, in their relationship to the diseases which they produce in human beings.

These viruses have been presented in the section on arthropod borne encephalitides, whereas, they might as well have been presented before the section on yellow fever, dengue, and sandfly fever. If yellow fever had first been encountered as a result of studies by inoculation of certain numbers of mice, we might have thought that we were dealing with a potential arthropod borne encephalitis in human beings. The important thing to remember and the thing that I, at least, am trying to keep in mind for my own orientation is that the diseases which are primarily and essentially viscerotropic in human beings can be almost essentially neurotropic in mice. The examples of yellow fever and dengue are always before us, and it is not at all improbable that perhaps, may never illnesses of a nature

On the other hand, it is not at all improbable that sometime or other we may find ourselves with an epidemic of encephalitis in human beings which would be caused by one of these viruses.

The fact of the matter is that many of these

#### IV VIRUS AND RICKETTSIAL DISEASES

by intracerebral or intranasal inoculation. It attacks certain cells in a specific manner and therefore appears to belong in the group of strictly neurocytotropic viruses despite the fact that the clinical picture caused by it is not characterized by marked neurological symptoms clearly in the same group with St. Louis and Japanese B encephalitis viruses. Semliki Forest and Bunyamwera viruses, having broader bases of pathogenic action, appear to be similar to the equine encephalomyelitis viruses, while the Mengo virus appears to belong to a group of which the prototype is poliomyelitis virus.

The observation that wild animals, notably primates, exhibit specific antibody against the viruses, suggests that each of them may involve these animals in natural epidemiological cycles, as in the case of the equine encephalomyelitis and yellow fever. The modes by which the agents are transmitted among human beings and animals are not known.

The paucity or the complete lack of knowledge concerning the clinical manifestations of the infections these viruses induce in humans places us in the position of having discovered the etiologic agents of several little known or unknown diseases. Thus, together with the fact that four of the viruses were discovered quite by accident gives reason to anticipate that Central Africa will remain for some time a fruitful field for virus research.

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Arthropod-Borne Virus Encephalitides," but for the term to define the group as it was meant to, all the words are essential. "Arthropod borne encephalitides" would include trypanosomiasis and other diseases, "Arthropod borne virus encephalitides" would be restricted to the group under discussion.

Dr K C SMITHURRY (Uganda). What Dr Hammon has just said covers part of my answer to Dr Sabin's first comment. I chose the title of my paper but I did not choose the name of the session, and have never maintained before, nor do I now, that any of the viruses that I have discussed are arthropod borne. I stated I did not know how any of them were transmitted. As a matter of fact, we have made experiments on insect transmission without success. The fact that three of these viruses have been isolated from mosquitoes is tentative evidence that mosquitoes can transmit them.

As to Dr Sabin's second comment on the relation of Mengo encephalomyelitis virus to poliomyelitis, I think the evidence is adequate that they are related, but if he would like to have it placed in some other relation than that which I have suggested, I have no objection.

viruses produce lesions in the spinal cord with a predominant on the anterior horn cells. I have reported before the American Society of Tropical Medicine that mouse adapted dengue virus produce a paralytic disease in monkeys indistinguishable in its manifestations from poliomyelitis, not only from the fact that the lesions in the anterior horn cells, but the fact the cerebral cortex is spared. The way to differentiate this group of viruses is not alone by the presence or absence of lesions in the spinal cord but by whether or not certain parts of the cerebellum are affected. The very fact that virus produces lesions in the spinal cord of monkeys does not mean that its prototype is poliomyelitis, because the same lesions are produced by dengue, yellow fever, and certain encephalitis viruses. I would like to mention the virus which Dr Jungblut has chosen to call Columbia SK virus and one studied by Dr Warren and Dr Smadel namely, the virus of encephalomyocarditis. It is perhaps, not improbable that when cross immunological studies are made, they may be found to be similar to, if not identical with, the mingo encephalomyelitis just reported by Smithburn. I wish to put in again this word of caution about classifying viruses in the poliomyelitis group simply because they produce lesions in the anterior horn cells of the spinal cord of mice or monkeys.

I cannot leave this discussion without expressing my great admiration and respect for the great addition to our knowledge of neurotropic viruses made by Dr Smithburn and his group in Africa during the past 10 years.

Dr GEORGE W. A. DICK (Uganda) I have very little to add to what Dr Smithburn has said about Mingo encephalomyelitis virus. As far as the clinical disease is concerned, this virus produces encephalitis in man. As Dr Smithburn said, in animals it produces lesions in the spinal cord not unlike those of poliomyelitis and we do know that it produces lesions in the cerebral cortex and in the mid brain of certain animals. During convalescence of my infection with this virus I developed marked immunity to it. We found immunity in monkeys which were in the open runs of the compound. The thing that surprised us slightly, though we haven't tested many human sera or antibodies (the total number is 140), is that we have found only 2 with serum antibodies and these came from the Budongo Forest area. Dr W. McD. HAMMOND (United States) I must have covered my subject so completely that no questions had to be asked, but I have more word that I would like to say in regard to the title of this session "Arthropod Borne Encephalitides." I believe Dr Reeves coined and used a term something like this "The Arthropod Borne Virus Encephalitides." It has gotten shortened in the publication of this program, and I would like to put forth an objection in regard to that shortening. I know that the words are rather clumsy,



The efficacy of dog control regulations for the elimination of rabies of the urban type has been demonstrated on numerous occasions. The

of dog control regulations in all infected regions. Rabies was again introduced into England in 1918 by one or more dogs which were illegally imported, but prompt application of dog quarantine regulations limited the spread of the disease, and by 1922 Great Britain was again free of rabies (3).

In countries where rabies is established in wild animals, the control of the disease depends on reduction of the number of known vectors in those regions where the disease is found to exist. Depending on the type of vector, this may or may not result in eradication of the disease. For example, if rabies is established in foxes, it can persist only if there is an abundance of these animals distributed over a large area so that the disease may migrate. In any one focus of infection, the number of animals unless the disease can be leads one to suspect the wild life which, because seldom seen by man

which persist in the absence of what we are apt to call the natural vectors of rabies. One of these foci is located in South Africa. In Transvaal, Orange Free State, and Cape Province, a variety of small wild carnivora belonging to the family Viverridae has been found to be infected with rabies. The yellow mongoose (*Cynictus penicillata*)  
 1 livestock,  
 t (*Genetta*  
 ), wild cat  
 have been

found to harbor the disease. Canine rabies has been controlled effectively in South Africa by dog quarantine regulations, and a campaign of destruction of the known vectors where outbreaks have occurred has reduced the incidence of rabies in man and domestic animals, nevertheless, there appears to be little chance of eradicating the disease in this locality (4).

In Mexico and South America, rabies is established in vampire bats in many regions, and the host virus relationship in this case is what one might expect in a true enzootic focus of rabies. Vampire bats are found only in Mexico and in Central and South America, and the principal vector of rabies has been identified as *Desmodus rotundus murinus* Wagner (5). The existence of rabies in vampire bats was recognized first in the State of Santa Catharina in southern Brazil. A paralytic disease of cattle and other livestock appeared in epizootic proportions in that region in 1908. When it was found that some of the diseased cattle were infected with rabies virus, a vigorous

## METHODS OF RABIES CONTROL

HAROLD N. JOHNSON *Laboratories of the International Health Division, The Rockefeller Foundation, New York City*

There are two epidemiological types of rabies, namely, the natural disease as it occurs in wild animals and the urban type, which is maintained in domestic dogs. The early history of rabies in Europe indicates that the disease was enzootic in wild animals in certain densely

spreading through factors masters from domestic dogs eventually became sufficiently numerous in all urban centers throughout the world to maintain the disease once it was introduced

teeth of the animal similar to snake venom but acting more slowly and that the disease could be prevented by local treatment of bite wounds. Experimental transmission of rabies from one animal to

prompted by the alarming frequency of human infection with rabies in Prussia. For example, from 1800 to 1810 there were from 200 to 300 human rabies deaths each year and the incidence then increased until 1819 when 356 human rabies fatalities were reported. Rigid dog control measures were then introduced and canine rabies became relatively rare in Prussia. By 1815 it had been eradicated and

remained so to the present time. The danger of reintroduction of the disease by importation of dogs was recognized and strict quarantine regulations were enforced to prevent it. Though dog control regulations eliminated rabies from many urban communities in continental Europe, these communities frequently became reinfected. The success of rabies-control work in one state was constantly jeopardized by the lack of similar action in adjoining states as well as by the widespread prevalence of rabies in foxes and other wild animals (1, 2)

Coast States of Mexico since 1910, is caused by rabies virus transmitted by vampire bats of the species *Desmodus rotundus murinus* Wagner. The virus isolated from the salivary glands of vampire bats captured in Mexico is closely related to known varieties of rabies virus as shown by cross neutralization, cross complement fixation and cross protection tests (13, 14, 15).

At intervals, rabies has occurred in epizootic proportions in wild animals in the United States of America. For example, the disease is known to have been epizootic in foxes in Massachusetts during the first decade of the nineteenth century, in Alabama in 1890, and in Alaska in 1915. These outbreaks were self limiting, at least as regards foxes, and there was no clinical evidence of persistence of the disease in other wild animals. Rabies is known to have been prevalent in the skunk species (*Spilogale putorius*) in Kansas for a period of several years, beginning in 1873. The presence of rabies in this species was recognized because of the occurrence of at least 40 cases of rabies in cowboys and hunters, who had been bitten by rabid skunks when camping on the plains. Another outbreak of skunk rabies was identified in Arizona from 1907 to 1910 because several persons developed rabies following skunk bite. In 1915 and 1916 rabies appeared in epizootic proportions in wild animals in California, Oregon, and Nevada.

Among the most important wild animals, coyotes were very abundant over a large region, and the disease persisted for many years despite an extensive campaign to reduce the number of known vectors. Since 1940 rabies has again

identified in foxes in 16 other States (16). The reported cases of rabies for the United States in 1946 included 8,384 dogs, 455 domestic cats, 1,055 cases in other domestic animals, and 906 cases in wild animals.

and 26 in man. The majority of wild animals found to be infected with rabies in the United States belong to the grey fox species (*Urocyon cinereoargenteus*), but the disease has been identified in the coyote (*Canis latrans*), skunk (*Spilogale putorius*, *Conepatus mesoleucus*, *Mephitis mephitis*), weasel (*Mustela cicognani*), wild cat (*Lynx rufus*), tree squirrel (*Sciurus carolinensis*, *Sciurus niger*), ground squirrel (*Citellus tridecemlineatus*), and muskrat (*Fiber zibethicus*). The disease has been identified entirely on laboratory

program of quarantine and destruction of dogs and cats was enforced because of the identification of sporadic cases of rabies in dogs (6). The persistence of the disease in cattle in the absence of any further evidence of rabies in dogs and cats suggested the presence of rabies in wild animals. The exact nature and method of spread of the disease was not clear until 1921 when Haupt and Behaag reported the isolation of rabies virus from a bat captured while feeding on a cow in daytime and established beyond doubt that the paralytic disease in cattle was caused by infection with the same virus (7). Torres and Lama later identified the vampire bat, *Desmodus rotundus*, as the source of the disease.

ch was diagnosed as  
 were four more cases  
 of ascending myelitis, and from one of these, brain tissue was submitted to the Rockefeller Institute in New York and the Lister Institute in London. Rabies virus was isolated from this material by workers in both laboratories. There had been no canine rabies in Trinidad since 1914, and stringent quarantine regulations had been kept in force to prevent its importation. Furthermore, there had been no cases of suspected rabies in dogs coincident with the outbreaks of paralysis in cattle (9). In 1936 Pawan reported the isolation of rabies virus from the salivary glands of vampire bats of the species *Desmodus rotundus murinus* Wagner, captured in Trinidad. He found that the rabies virus isolated from these bats was related to a  
 and confirm  
 vampire bat could transmit rabies as a symptomless carrier (10, 11). An investigation revealed that 55 persons had developed paralysis and died after being bitten by vampire bats. A program of destruction of bats of all species was initiated, and the disease soon disappeared (12). It has been shown recently that a paralytic disease of livestock called derriengue, which has been prevalent in the Pacific

product may be classed as a live virus vaccine as the infectivity of the fixed virus is maintained for several months when treated in this manner and stored at 4° C. The experimental studies of Umeno and Doi showed that dogs given 5 cubic centimeters of this vaccine by subcutaneous injection would develop a good rate of immunity to challenge with street rabies virus given by intraocular injection. This work was confirmed by F. Blakeslee and J. E. Smith (21). The vaccine was found to be stable for at least 1 year.

In areas, and in communities where rabies control was limited to vaccination of all dogs allowed at large, the disease disappeared. One possible case of rabies caused by the vaccine virus was observed in approximately 30,000 dogs immunized in Japan, and in the United States it was observed that in extremely rare instances the vaccine could produce infection with the vaccine virus (22). This led to the ruling by the United States Department of Agriculture that rabies vaccine used for immunization of dogs must contain no active virus as determined by intracerebral test inoculation in rabbits. The fear of spreading rabies by vaccination of dogs with active fixed rabies virus was unjustified, in that this variety of virus does not propagate in the salivary glands and is not found in the saliva, furthermore, the disease produced in this way is uniformly of the paralytic type.

In order to meet the requirement of safety tests, commercial laboratories prepared a canine vaccine of the Semple type in which the concentration of brain tissue was increased to 20 percent. It is well to note that the 5 cubic centimeter dose of this vaccine recommended for immunization of dogs contains approximately the same amount of virus as the 10 cubic centimeter dose of the Umeno and Doi vaccine.

its capacity to immunize (23, 24, 25, 26). Recent studies have shown that this type of vaccine is an effective immunizing agent when tested in dogs, and a single subcutaneous injection of 5 cubic centimeters of the concentrated brain antigen of the Semple type vaccine produces a high grade of resistance to challenge with rabies street virus, provided that the exposure approximates that received in nature. This can be accomplished by intramuscular test inoculation with virus derived from infected salivary gland tissue. The immunity produced by this method of immunization persists at a satisfactory level for at least 1 year, but three doses of vaccine, in 5 cubic centimeter amounts, given a week apart, produced a more certain immunity to rabies (27). The development of a mouse test for assaying the potency of rabies vaccine has resulted in improvement in vaccine production, and rabies vaccine distributed by commercial biological laboratories in the United States for immunization of man and lower animals must pass a prescribed test. This test, which was developed by Webster (28) and standardized by Habel (29), consists of titra-

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do not reveal the true extent of the disease. The current high prevalence of rabies in wild animals in the United States is clearly attributable to factors which have allowed wild animals, particularly foxes, to become abundant over a large area. To a certain degree, the population of foxes was brought about by game protection regulations, but it is well known that wild animals increase and decrease in a cyclical manner, and all one can say is that conditions have been very favorable for foxes.

Let us now examine the problems encountered in attempting to eliminate the urban type of rabies, which is the major source of human exposure. The object here is to prevent any dog from biting another for a period of the longest latency of the disease, and this can be accomplished by quarantine of owned dogs and elimination of stray ownerless dogs. In order to maintain a quarantine of owned dogs, it is necessary to deal with dog owners, and in a country such as the United States where almost every family has one or more dogs, they are in the majority, and no dog-control legislation can be enacted or enforced without due process of law. Experience has shown that registration and licensing of dogs is one of the most important features of a dog control program. It is proper that dog owners be taxed to provide dog pounds and personnel to eliminate ownerless stray dogs and enforce dog restrictions or quarantine according to necessity. Since such basic dog control provisions are lacking in some sections of the United States, it has been impossible to eliminate the urban type of rabies completely by application of dog-quarantine regulations. The presence of rabies in wild animals and the failure to deal effectively with the urban type of the disease by means of quarantine regulations has encouraged the investigation of immunization of dogs as a method of rabies control.

Pasteur demonstrated that dogs could be made refractory to intracerebral challenge with street rabies virus by repeated subcutaneous injections of fixed rabies virus (18). Because it was necessary to give a series of inoculations to make dogs resistant to this challenge, it was concluded that the same would hold true for natural exposure, and it did not appear practicable to immunize dogs as a method of rabies control. The failure to recognize the artificial character of intracerebral inoculation as a test of immunity retarded the development of a satisfactory method of prophylactic immunization of dogs to rabies.

In 1921 Umeno and Doi introduced a single injection method of vaccination for dogs, which proved to be very effective for the prevention of rabies in naturally exposed animals (19, 20). The vaccine developed by these men was patterned after the Fermi phenol treated, fixed rabies virus vaccine, but the concentration of brain antigen was increased to 20 percent, and glycerol was added as a preservative. The chemically treated virus was exposed to room temperature for 2 weeks, was held at refrigerator temperature 1 month before use. This







fifth case I shall now deal with along with the sixteenth case during the 1919-39 period referred to above. In the latter case the 6 months quarantine was up on the 1st of November 1929 but the dog remained in quarantine for a further period at the owner's request, it developed rabies 6 months and 21 days after arriving in Britain. In 1947 a similar circumstance arose, a dog was imported from Italy on the 30th of September 1946. It was not removed from quarantine by the owner and it developed rabies on the 24th of May 1947, i.e., 8 months after its arrival in the country.

The experience of the Ministry justified the 6 months' quarantine quarantine is based upon the period may be longer even than 6 months, but the cases in which the incubation period exceeds 6 months are very exceptional. It is not considered practicable to legislate for these exceptional cases, but it is of course realized that there is still a risk, as the records to which I have

have happened if the dogs in quarantine which developed rabies 7 months and 8 months, respectively, after arriving in Great Britain had been released at the regulation limit of 6 months—implies that even in a country where such measures are proceeding to provide may arise with a spread of human beings. This work should include the institution of more rapid and accurate modern methods of diagnosis and the preparation and standardization of vaccines, for human beings, of more satisfactory character.

be developed for protecting human beings against rabies by vaccination. It may be that ultraviolet irradiated virus may provide an immunizing agent for more universal adoption, and further advances

alone may be sufficient to diminish the risk of introduction and spread

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adequately the present state of our knowledge on the subject of methods of control of rabies.

It is clear, and Dr Johnson has explained why, that the polio must vary according to the conditions obtaining in the country district concerned. Some countries are more favorably placed geographically than others and in them it is easier to prevent the introduction of the disease and to limit its spread and the possibility of its becoming endemic should the regulations in force break down.

The British Isles among other countries are so placed but, while such a country may be regarded with envy by its less fortunate neighbours, that is no reason why its endeavours should not be fully comprehended and if at all possible imitated, at least to some degree. I have already covered some of the ground in a review in the *Tropical Diseases Bulletin*, 1915 (vol 42, p 680-682), and I wish specially to stress some facts of interest with regard to quarantine measures as a method of diminishing the risk of introduction and spread of the disease. During the period 1887 to 1893, 11 years, there were 2357 cases of rabies in animals. Regulations based on the quarantine of imported dogs and cats which since 1897 have become more stringent have produced a most satisfactory result. There were 46 cases between 1897 and 1902, 6 years, and between 1903 and 1917 there were none. During the latter half of the nineteenth century the annual human deaths from rabies in Great Britain increased to the alarming total of 70 in 1877. Since 1898 only two deaths from hydrophobia have been recorded.

The regulations in force require all canines and felines landed in Great Britain from abroad to be quarantined for a period of six calendar months after landing on premises approved by the Ministry of Agriculture. Now, some may imagine that such a rule is overcautious and perhaps unnecessary.

The disease was introduced once only since 1917 into Great Britain. This was due to the illegal importation of a dog or dogs. The consequences were very serious. From 1918 to 1922 there were nearly 325 cases of rabies found in animals and there were nearly a thousand suspected cases reported. Three hundred and fifty-eight persons were bitten, 123 of these by dogs known to be rabid, and 144 people received treatment. Since 1922 in Great Britain outside quarantine kennels no case of rabies has been confirmed since 1922. During the period 1917 to 1939 21 years there were 16467 dogs quarantined and 16 of these developed rabies. In six of these cases disease developed from 1 to 12 days after the dog was landed in country, in five the disease developed between 1 month and 3½ months after the dog was landed and in four cases between 4 and 10 months. The sixteen cases I shall refer to later. During the 1918-4000 dogs were quarantined and 5 developed rabies, one in 1918, two in 1919, and a fourth 10 weeks after landing. The

automobile in the past 10 years came down with rabies after they arrived in Colorado and started small foci of urban rabies. Two resulted in the deaths of two human beings. The question arises, and we are facing the problem now in Denver, Colo., as to how to bring about an inter State system of isolation or regulation of this problem.

Dr J H S GEAR (Union of South Africa) As Dr Johnson mentioned, rabies in South Africa is most commonly transmitted by the bite of the yellow mongoose or meerkat.

The history of the cases is often the same. It is a common pastime for children to chase meerkats. If the meerkat is healthy it cannot be overtaken. If, on the other hand, the meerkat is sick it may readily be caught. In such cases the child is bitten and the meerkat very often is bitten.

A week or later the child

me why canine rabies is so rare in dogs in South Africa, because dogs often also chase and catch meerkats. Perhaps Dr Johnson would tell us whether any antigenic or host susceptibility difference would account for this fact.

The last case of rabies in which I was directly concerned was a case in a South African soldier. In August 1915 he was at a football match in Cairo and became involved in a dog fight (between two dogs). He was bitten. The dog was captured and observed. It was reported that it had remained healthy. However, in January 1916 the soldier died of rabies. Is a silent infection of a dog with rabies possible, or is

mission by vampire bats in South America and the vampire bat, *Viverridae* in South Africa. In East and Central Africa, the jackal is usually the first animal in which cases are observed. The disease is then transmitted to domesticated canines and other domesticated animals.

The disease in East Africa is associated with forest areas, particularly with the group of forests which formed part of the equatorial forest belt many years ago. For the last 30 years, the infection has never traveled very far from this belt and the disease has never reached Nairobi or the country between Nairobi and the coast. There is as other speakers have emphasized, still much to be discovered about rabies in wild animals.

With regard to vaccination, I have always taken the view, in dealing with a country of which only a part was infected, that if vaccine was going to be regarded as an alternative to quarantine measures, then one should be very cautious about the adoption of vaccination as a prophylactic.

Dr J H S GEAR (Union of South Africa) Dr Johnson mentioned that in Argentina

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to a minimum. In others where the disease is continuous in the United States combined methods which may include vaccination and quarantine of dogs as well as destruction of rabid animals would have to be applied. It is obvious that not be placed on vaccination of dogs alone, especially as this in some minds to a false measure of security and make it apply quarantine and other measures.

With regard to the disease occurring in South Africa in vampire bats in South America, in skunk and foxes America, I was wondering if Dr Johnson would say something the suggestion that there may be differences in antigenic strains of rabies virus. There were reports of differences of behavior between strains of virus of canine origin and associated with the disease as transmitted by other species not possible that some antigenic differences did exist and that those that these could not always be demonstrated is due in some to laboratory procedures, such as passage in and adaptation to a animal other than the one in which the disease occurred under natural conditions? It is known that modification of a virus may occur a result of passage through another species and it would appear arising if false interpretations are not made sometimes because such factors.

Dr A. L. Bruceño Rossi (Venezuela) Tenemos el problema complejo de la rabia y lo llamamos así porque existen dos tipos de virus diferente antigénicamente que hemos aislado de los perros, uno de esos virus idéntico al virus de la rabia bovina, por lo cual creemos que en casos desgraciados de sujetos vacunados con virus pasteur esos sujetos no fueron inmunizados y experimentalmente resulta lo mismo.

Los animales frecuentemente infectados en Venezuela son perros, gatos y ganado bovino. No se ha podido aislar virus rábico de zorros, así en los campos existen muchas personas con secuelas o estigmas de mordeduras de este animal (zorro) cuando invaden en época de sequía hacia las viviendas de los llanos. El problema de la vacunación antirábica debe estudiarse más a fondo y nosotros pensamos que la probable pluralidad de virus rábico hace sugerir la conveniencia de utilizar en los accidentes ocasionales para el hombre, una vacuna polivalente, es decir con virus fijo de tipo pasteur y bovino.

Dr EDWARD R. MOURAGE (United States) We have very little evidence of rabies among wild animals in the western part of the United States, particularly within Colorado, although we have picked up a few coyotes showing the disease. We have had epidemics of urban rabies and particularly one in 1927 when it was estimated that approximately 2,000 dogs died of the disease in and around Denver. I have a question to ask Dr Johnson with respect to control of urban rabies, which is a problem in the United States where we have 48 States with adjoining boundaries and no effective quarantine between them. It has come to my attention that four dogs which were

also spread the disease over large regions. The present wide distribution of rabies in the United States is largely due to the unrestricted transport of dogs from state to state by automobile.

The discussion has brought out some interesting points regarding the epidemiology of sylvatic rabies. The observation by Dr Daubney that there appears to be a reservoir of rabies among wild animals in the equatorial forest belt in Kenya, East Africa, is of particular interest. It was noted that Norway rats are occasionally found to be infected with rabies and it may be that the disease is established in this species in some urban centers. An attempt should be made to determine whether rats may carry rabies as an asymptomatic infection of the salivary glands in the manner of vampire bats.

(The session adjourned at 12 m.)

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ción ha desaparecido. Hubo un brote de rabia en la Provincia de Juan, muriendo 14 personas con 800 animales infectados. Por inspiración del Dr. Briceño Rossi de Venezuela estoy en el aislamiento de ambos virus en la Ciudad de Buenos Aires. Crivellari y Calabresse del Laboratorio Pasteur de Buenos hallan estudiando en estos momentos la neutralización de rabico por el bismuto. Usando bismuto soluble conseguimos proteger el 70% de los animales en el primer pasaje. Todas estas experiencias fueron comunicadas al 2º Congreso de Enfermedades Infecciosas, realizado en la República Argentina 1917.

Dr. ORTELLO MARTINES FORTY (Cuba). The first antirabies institute in the Americas was founded in Cuba by Dr. Santos Fernandez. In the last 10 years, the number of human cases of rabies has fallen from 0 to 4 cases per year in a population of 1,000,000.

Among the legal measures taken in Cuba, the owner of a dog must appear with the dog collected and after 8 hours, he pays a fine and his dog is vaccinated, if it was not previously vaccinated. Dogs who have bitten someone are observed for 14 days in official institutions or in their homes by veterinarians. If clinical symptoms of rabies appear, a telegram is sent to the bitten person and free vaccination is provided.

Dr. W. McD. HAMMOND (United States). I have nothing original to report, but I have just returned from the meetings of the Society of American Bacteriologists. Drs. Koprowski, Cox, and others reported serum prophylaxis. If a highly concentrated rabbit immune serum was administered in adequate amounts within 24 hours or less after inoculation with street virus in dogs and hamsters, protection was afforded to a high proportion of animals. This, though requiring further confirmation and extension to man, suggests that a useful agent may eventually be available for the prophylaxis of rabies.

Dr. HAROLD A. JOHNSON (United States). The data presented by Dr. Galloway concerning the development of rabies in dogs imported into Great Britain illustrate the ease with which this disease may be disseminated by domestic dogs. The very long incubation period of the disease in some animals makes it evident that the 6 months quarantine period required for dogs imported into Great Britain is justified. The presence of a water barrier about the British Isles limits the introduction of rabies to animals imported by plane or boat. In large continental regions, it is much more difficult to prevent the spread of the disease. Rabid dogs have been known to travel distances of more than 100 miles and wild animals such as foxes and wolves can

# NATURAL IMMUNITY AND SUSCEPTIBILITY OF DOVES AND PIGEONS TO EXOERYTHROCYTIC AND ERYTHROCYTIC STAGES OF *PLASMODIUM RELICTUM*<sup>1</sup>

CLAY G. HUFF, *Naval Medical Research Institute, Bethesda, Md*

## INTRODUCTION

The demonstration (Huff and Coulston, 1946) that preerythrocytic stages of *Plasmodium gallinaceum* and *P. relictum* could grow in the tissues of certain hosts following the inoculation of sporozoites even though parasitemia did not develop indicated that differences exist between the susceptibility of the hosts to erythrocytic parasites and their susceptibility to preerythrocytic stages of the same species. Their experiments (1946, 1947) with Courtney's (1938) pigeon strain of *P. relictum* (1P) and the strain adapted to canaries by Redmond (1P1) brought to light some interesting relationships between these strains and their avian and mosquito hosts. The 1P strain produced heavy and often fatal infections in pigeons but was incapable of infecting mosquitoes. After even one passage in canaries this modified strain (1P1) would infect mosquitoes (*Culex pipiens*) readily (Redmond, 1944). The sporozoites from such infected mosquitoes were capable of producing preerythrocytic stages in both pigeons and canaries. Although this tissue infection was followed by parasitemia in the canary, no parasitemia developed in the pigeon. The first step toward revealing the complex relationships between this parasite and its hosts was to test as many of the close relatives of the domestic pigeon as possible. This paper reports that experiments, together with a few observations in which species hybrids were used as hosts

## EXPERIMENTAL PROCEDURE

The birds used in these tests belonged to the family Columbidae to which the domestic pigeon also belongs. They were obtained from

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100. 101. 102. 103. 104. 105. 106. 107. 108. 109. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120. 121. 122. 123. 124. 125. 126. 127. 128. 129. 130. 131. 132. 133. 134. 135. 136. 137. 138. 139. 140. 141. 142. 143. 144. 145. 146. 147. 148. 149. 150. 151. 152. 153. 154. 155. 156. 157. 158. 159. 160. 161. 162. 163. 164. 165. 166. 167. 168. 169. 170. 171. 172. 173. 174. 175. 176. 177. 178. 179. 180. 181. 182. 183. 184. 185. 186. 187. 188. 189. 190. 191. 192. 193. 194. 195. 196. 197. 198. 199. 200. 201. 202. 203. 204. 205. 206. 207. 208. 209. 210. 211. 212. 213. 214. 215. 216. 217. 218. 219. 220. 221. 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2014. 2015. 2016. 2017. 2018. 2019. 2020. 2021. 2022. 2023. 2024. 2025. 2026. 2027. 2028. 2029. 2030. 2031. 2032. 2033. 2034. 2035. 2036. 2037. 2038. 2039. 2040. 2041. 2042. 2043. 2044. 2045. 2046. 2047. 2048. 2049. 2050. 2051. 2052. 2053. 2054. 2055. 2056. 2057. 2058. 2059. 2060. 2061. 2062. 2063. 2064. 2065. 2066. 2067. 2068. 2069. 2070. 2071. 2072. 2073. 2074. 2075. 2076. 2077. 2078. 2079. 2080. 2081. 2082. 2083. 2084. 2085. 2086. 2087. 2088. 2089. 2090. 2091. 2092. 2093. 2094. 2095. 2096. 2097. 2098. 2099. 2100. 2101. 2102. 2103. 2104. 2105. 2106. 2107. 2108. 2109. 2110. 2111. 2112. 2113. 2114. 2115. 2116. 2117. 2118. 2119. 2120. 2121. 2122. 2123. 2124. 2125. 2126. 2127. 2128. 2129. 2130. 2131. 2132. 2133. 2134. 2135. 2136. 21

## SECTION V

### Malaria

#### Session 1 PARASITE HOST RELATIONSHIP

*Monday, May 10—2 to 4 30 p m*  
*Departmental Auditorium, Main Hall*

The convener of section V, Dr Mark F Boyd, called the meeting to order and welcomed the delegates and members. He then conducted the election of a chairman and two vice chairmen. The elected and appointed officers of the section were as follows Maj Gen Sir Gordon Covell, United Kingdom, chairman, Dr Arnaldo Gabaldon, Venezuela vice chairman, Col M K Afridi Pakistan, vice chairman, Dr Mark F Boyd, United States secretary, Dr L Harold Hinman, United States assistant secretary.

A motion proposed by Dr Paul F Russell was adopted which empowered the chairman to appoint a special committee to consider the possibility of the permanent fusion of the Congress on Tropical Medicine with the Congress on Malaria. The chairman appointed the following as members of this special committee Prof A H Swollen, Grebel Dr Giulio Ruffale and Dr Carlos A Alvarado. The papers which follow were presented and discussed in the first and succeeding sessions of section V.



(*Phaps chalcoptera*) exhibited some degree of parasitemia on basis of microscopical examination of the blood. High parasitemias resulted from such inoculations in triangular spotted pigeons (*Columba guinea*), Senegal doves (*Streptopelia senegalensis*), dwarf turtledoves (*Streptopelia humilis*) and California mourning doves (*Zenaidura macroura*). Infection in ringdoves (*St risoria*) was moderately high. In only five instances were the infections suitable for infectivity tests on mosquitoes (*Culex pipiens*). No infection was found in any of the mosquitoes tested. Thus, whatever the inimical effect is that the blood of the domestic pigeon has on the infectivity of gametocytes for mosquitoes, this effect is also present in these five species of birds belonging to three genera.

In interpreting the results obtained from sporozoite inoculations, it must be emphasized that many factors may contribute to negative

number of failures to demonstrate preerythrocytic stages in a species of host known to be susceptible to this stage of the parasite. For this reason, and also because not more than two individual birds were examined in each species, the number of negative findings is probably not a true index of the susceptibility of this group of species to preerythrocytic stages.

In nearly all instances, however, a few sections were found containing mosquito scales and trachea which had been introduced in the inoculum, and one can reasonably assume, therefore, that sporozoites had been inoculated into the areas which were examined microscopically. The two species in which preerythrocytic stages were demonstrated microscopically were *Streptopelia senegalensis* and *S semitorquata*. Segmenters and large schizonts were found, respec-

skin, parasitemia was observed. Such examples have little signifi-

African turtledove, and Grayson's pigeon). Subpatent infections were demonstrated in three instances in which neither preerythrocytic nor erythrocytic stages were seen in the bird which had been inoculated with sporozoites, (triangular spotted and California bandtail pigeons, *C guinea* and *C fasciata*, and ringneck dove, *St risoria*). Two hybrids derived from crosses between male domestic pigeons (*Columba livia*) and female ringdoves (*Streptopelia risoria*) were tested in similar manner except that each individual was inoculated with sporozoites. No preerythrocytic nor erythrocytic stages were observed in these hybrids, and all canaries which were inoculated with

sporozoites were  
found

the tested pigeon Failure for *varicellum* existence of a subpatent infection in

TABLE 1.—Results of inoculations of blood (IP) and of sporozoites (IP1-1) of *P. relictum* in pigeons and doves

| Species                    |                                | Blood inoculations |                            | Sporozoite inoculations |               |           |
|----------------------------|--------------------------------|--------------------|----------------------------|-------------------------|---------------|-----------|
| Common name                | Scientific name                | Parasitemia        | Indicating for sporozoites | Time, days              | Parasitemia   |           |
|                            |                                |                    |                            |                         | Microscopic   | Subpatent |
|                            |                                | +(20%)             | —                          | —                       | —             | +         |
|                            |                                | +                  | —                          | —                       | —             | +         |
|                            |                                | +(12 p. h. f.)     | —                          | —                       | —             | +         |
|                            |                                | +(22 p. h. f.)     | —                          | —                       | —             | +         |
|                            |                                | +(10%)             | —                          | +                       | +             | +         |
|                            |                                | +(10%)             | —                          | +                       | (10%)         | +         |
|                            |                                | +                  | —                          | —                       | +             | +         |
|                            |                                | +(10%)             | —                          | —                       | (12 p. h. f.) | +         |
|                            |                                | +(15%)             | —                          | +                       | —             | +         |
| Dwarf turtle dove          | <i>Streptopelia dussumieri</i> | +                  | —                          | —                       | +             | +         |
| California mourning dove   | <i>Streptopelia macroura</i>   | +                  | —                          | —                       | +             | +         |
| Grayson's pigeon           | <i>Columba graysoni</i>        | +                  | —                          | —                       | +             | +         |
| Australian crested         | <i>Oryzopsis leucotis</i>      | +                  | —                          | —                       | +             | +         |
| Malacca white-wing         | <i>Macropygia tenuirostris</i> | +                  | —                          | —                       | +             | +         |
| Pouter-wing pigeon         | <i>Columba palumbus</i>        | +                  | —                          | —                       | +             | +         |
| Vienna White Pouter        | <i>Columba palumbus</i>        | +                  | —                          | —                       | +             | +         |
| Hybrid (2 F <sub>1</sub> ) | <i>Columba palumbus</i>        | +                  | —                          | —                       | +             | +         |

<sup>1</sup> Subpatent parasitemia was demonstrated by the appearance of parasitemia in sub-inoculated recipients.

<sup>2</sup> p. h. f. indicates number of parasites per 100 microscopic fields.

<sup>3</sup> Subinoculated birds died before time for parasitemia to appear.

The results of these tests are as follows:

which they were found (Senegal dove and dwarf turtledove) the

only a single parasite was found on microscopical examination

When the results are considered as a whole, it would appear, in spite of the small numbers of animals employed, that the series contains species with a wide degree of susceptibility to *P. relictum*. Two species (Australian crested dove and bronzewing pigeon) failed to exhibit any evidence of infection when inoculated either with infected blood or with sporozoites. On the other hand, Senegal doves acquired a high parasitemia from the inoculation of blood and also acquired tissue and blood infections from the inoculation of sporozoites. Between these two extremes, other degrees of susceptibility were represented by the other species, and previous work (Huff and Coulston,

\*) result

rosses are

ages can

not observed

likewise more like the dove parent

The characteristic of a host which we call natural immunity (or the opposite or complex one) may influence

possibly be factors having no relation to inheritance. The first step in the analysis of this complex characteristic here reported has shown that some pigeon and dove relatives of the domestic pigeon exhibit

pigeons are in progress

#### REFERENCES

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 Coulston F and Huff C G. *J Infect Dis* 80 209-217 1947  
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blood from the hybrids died before the results of the subinoculation could be ascertained. Thus, the reaction of the two hybrids was similar insofar as it was determined, to the female parent, ringdove.

## V MALARIA

### DISCUSSION AND SUMMARY

The inoculation of a series of hosts belonging to different species by two methods, that is, by infected blood and by sporozoites, presents a comparison of the efficacy of the two methods for establishing a strain of malaria in new hosts. The results here obtained clearly indicate that in general it is easier to establish a malarial infection in a new host by inoculating infected blood than it is by inoculating sporozoites. This principle was previously illustrated in the behavior of the same strains of parasites in domestic pigeons in which a transfer of infection by blood inoculations from infected to uninfected pigeons was effected easily, whereas little or no parasitemia resulted from the inoculation of pigeons with sporozoites.

It is questionable whether the relative degrees of parasitemia which resulted in different species of pigeons and doves from inoculation of infected blood can be related to the degree of consanguinity of the hosts. The experiment would need to include a larger number of animals before any real significance could be attached to the different degrees of parasitemia in the various species. In this respect, it is of interest to note that the highest and a very low degree of parasitemia resulted in *Columba guinea* and *Columba fasciata*, respectively.

It is significant that in the infections obtained by inoculation of infected pigeons blood the gametocytes in all five of the species tested were unable to produce infections in mosquitoes. Since this is the case and since a similar transfer to canaries yields gametocytes capable of infecting mosquitoes it would appear to be likely that some substance in the erythrocytes or plasma of domestic pigeons and also in other closely related birds is inimical to the production of viable gametocytes. An alternate explanation could be that a substance essential to production of gametocytes is present in the blood of canaries and absent in the blood of the species of pigeons and doves tested for and investigation of the nature of this substance is in progress.

The small number of preerythrocytic stages found in the series of hosts inoculated with sporozoites has some significance in spite of the difficulties already mentioned in regard to demonstrating these stages even in a known susceptible host. These stages were demonstrated in a large percentage of the domestic pigeons which were used previously (Huff and Coulston, 1916, Coulston and Huff, 1917). Prolonged searching was made for the preerythrocytic stages in sections of the biopsied areas of skin taken from the birds in the series. Such extensive searching was not necessary in our studies on the domestic pigeon. Even in the two species in

Later, Iyer, Shortt and Menon (1941) described forms earlier in the incubation period than those noted in the previous communication. Owing to the existing state of war, these findings were reciprocally unavailable, and the same thing occurred in the case of the work next to be noted.

Reichenow and Mudiow (1943) next gave a clear account of the development of the pre erythrocytic stages of *P. relictum* following the inoculation of sporozoites into the tissues of canaries. This was followed by the classical paper of Huff and Coulston (1944) which gave a still fuller account of the same process in *P. gallinaceum*. Here again, war conditions prevented reciprocal access to the work of the respective authors so that full credit must be given in each case.

These two papers were so great an advance on previous knowledge, as showing the early stages of development, that I think there has been a tendency to presume that the highly artificial conditions of the experiment represent what occurs in nature. By this I mean that much greater numbers of sporozoites were introduced into a localized area of the skin than would ever occur in nature. The development of the parasite subsequently studied in this area, represented that

flushed in the spot by entry into those attracted to the area. It carried further afield to other

parts of the reticulo endothelial system, such as brain, liver, spleen etc., would develop there, and it is even possible that the local development at the site of the inoculation would be unusual rather than

than the  
ectly into

the brain will develop there producing stages similar to those described by Huff and Coulston. Working with B. Malamos, the author has also found early developmental stages (at 48 hours) in the brain of infected mosquitoes. So did not seem capable of its of assiduous work in

various parts of the world—certainly in America, Europe and Asia.

We now come to the work of Fairley (1945) and his collaborators in Australia. Fairley inoculated very large quantities of blood from volunteers bitten by mosquitoes infected with both *P. vivax* and *P. falciparum* into other volunteers. He found that if the volunteer was bitten on one arm, the blood taken from the opposite arm was infective to the second series of volunteers from 7 minutes after the

1 hour afterwards  
ilation into volun  
and 9 days in the  
lays on which the

parasite became infective on reaching the circulation. From these experiments, one could not escape the inference that the intervening

## THE PRE-ERYTHROCYTIC CYCLE OF *PLASMODIUM CYNOMOLGI*

H E SHORTT, M D, D Sc, D T M & H, *Professor of Medical Protozoology, University of London, and Director, Department of Parasitology, London School of Hygiene and Tropical Medicine, Great Britain*

I do not propose to go in detail into the past history of attempts to find the pre erythrocytic stages of malaria parasites as it would be superfluous before an audience as instructed as this. In order, however, to lead logically to the present position of our knowledge of these forms, I shall chronicle, in correct sequence, the more important results achieved by workers in this field up to the present time and then proceed to a description of my more recent work.

history of the malarial parasite during the incubation period of malaria. Raffaele (1931) was probably the first to describe other than erythrocytic forms of malaria when he described stages of *Plasmodium elongatum* in the bone marrow of birds. Huff and Bloom (1935), who also described certain forms of *P. elongatum* in cells of the bone marrow, considered this to be precocious invasion of erythroblasts. In 1937 Raffaele reported the finding of unpig-

life cycles of all

Kikuthi and Mudrow (1939) described early stages of *P. cathemerium* after injection of sporozoites into the pectoral muscles of birds. These forms were seen 16 to 40 hours after the inoculation. Casini

cular injection of sporozoites. In the same year Brug (1940) reported the finding of intracellular unpigmented parasites in the lung of a case infected with *P. vivax* by blood inoculation.

EXPLANATION OF PLATES

Pre-erythrocytic Development of *Plasmodium cynomolgi* in the liver cell

Figure 1.—Fifth-day stage.

Figure 2.—Sixth-day stage.

Figure 3.—Seventh-day stage—entire form.

Figure 4.—Seventh-day stage showing indentation.

period of noninfectious blood represented the time taken for development of the sporozoite into the erythrocytic form of the parasite in some site protected from the circulation or, if in the circulation, in an uninfected form

### MATERIALS AND METHODS

A large colony of *Anopheles maculipennis atroparvus* was maintained as the vector mosquito, and the parasite used was *Plasmodium cynomolgi* in monkeys of the species *Macaca mulatta*.

We commenced a series of experiments on lines which must have been duplicated wherever the same search for pre erythrocytic stages was going on, i. e., infection of mosquitoes from a gametocyte-carrying monkey and the refeeding of these on clean monkeys. The latter were then examined at various intervals of time between infective feed and the appearance of erythrocytic forms in the blood, which was about the ninth day.

We were fortunate in being able to synchronize the breeding of a large number of mosquitoes with the production of good gametocyte carrying infections in the monkeys. The details of the experiment as the result of which pre erythrocytic forms were first discovered, are briefly as follows.

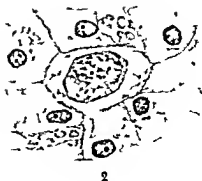
A rhesus monkey (*Macaca mulatta*), showing gametocytes of *P. cynomolgi* in the blood, was fed upon by about 1000 *Anopheles maculipennis atroparvus* bred in the laboratory. These were given a subsequent feed on another gametocyte carrying rhesus monkey and a third feed on the monkey first mentioned. The fed mosquitoes were maintained on raisins and cube sugar at a constant temperature of 26° C and in a relative humidity of about 80 percent. Ten days after the third infective feed, 20 mosquitoes dissected all proved heavily infected. The survivors, 576 in number, were now given the opportunity to feed on a clean rhesus monkey. Over 500 fed. The total number of mosquitoes was now ground up in a mortar in about 8 cubic centimeters of heparinized monkey plasma diluted with normal saline solution and the suspension, one half intraperitoneally and the other half intramuscularly, inoculated into the same monkey. The monkey was brought to post mortem on the seventh day after infection and its tissues and internal organs examined for the presence of pre erythrocytic forms. Although the examination is by no means complete even now, it may be said that pre-erythrocytic forms have so far been



Figure 5 —Seventh-day stage showing two vacuoles

Figure 6 —Seventh-day stage—multivacuolate

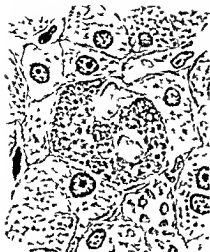
Figures 7 and 8 —Seventh-day stages showing lobate arms



*Pre erythrocytic development of plasmodium cynomolgi in the liver cell*

Figure 9.—*Eighth-day stage.*

Figure 10 —*Ninth-day stage.*



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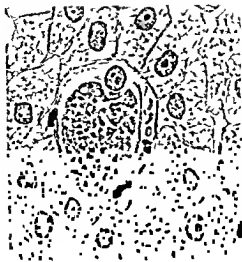
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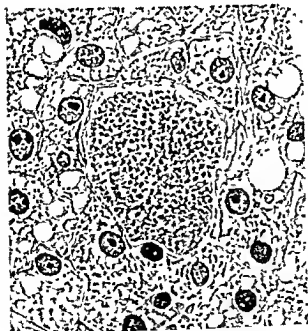
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Figure 11.—Tenth-day stage showing recently ruptured schizont with merozoites escaping; two phagocytic cells in the center.

Figure 12 —Incision of ruptured schizont by phagocytes.

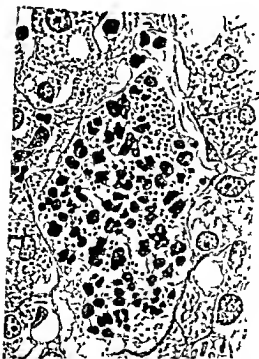


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monkey and in such cases we were able to follow the same parasite through its early and later stages. When it came to the rupture of the schizonts which took place about the eighth, ninth, and tenth days, my mere mention of these days shows it was not synchronized. If one took the tenth day as a base line one would find quite a large number of schizonts still unruptured but with some merozoites formed, others newly ruptured, others which had probably ruptured the day before and where the phagocytes had removed most of the merozoites, so that one might say the stage of maturity might be extended over a period of perhaps 3 days and the synchronism therefore was not strict, by any means. In other words, the blood cycle had already been established while in the same animal the pre erythrocytic development was going on in other parasites.

I wasn't quite sure of the question Professor Raffaele asked me. I was very glad to see him because just before the war he very kindly took me over his own laboratory and showed me the work which he

anything earlier than the fifth day. Well, we haven't done so. I feel that if one were to examine sufficiently long the sections of liver from a heavily infected monkey one ought to be able to find at least a fourth day form. Those seen on the fifth day are already 11 or 12 microns in diameter. The earlier ones must be considerably smaller but if we search long enough we would find them, although we have actually not done so.

Brigadier Sinton asked me to say a few words on the question of vivax. Well, that really was a small step after the present one. *Cynomolgi* and *vivax* are so similar that we felt sure the human form would show the same development as in the monkey. The actual experiment was carried out in the same way. We first of all fed 3,600

14 days after the last feed, the second feed, that we fed these to human volunteer. Now on this human volunteer we fed 2,010 of these infected anopheles. It took all day to do it. In fact, it took 2 days to do it. In addition to that, 200 of the anopheles had their salivary glands removed and these were inoculated intravenously into the same individual. Here again it was taken without a murmur and there were apparently completely innocuous effects. A piece of the liver from this human volunteer was removed on the seventh day by operation, quite a large piece, about as big as the end of my thumb—and that again is a simple operation. It seems to be without any great danger as our experience with *Cynomolgi* was. We were then able to find the pre-erythrocytic stage of *Cynomolgi* in the liver, but I think

## V MALARIA

and fever occurred in one case on the fifth day, and in another case parasites occurred on the fourth day.

There is therefore no doubt that in human malaria as well as in avian malaria the penetration of parasites into the blood does not take place for at least 4 days after inoculation of sporozoites.

Dr CLAY G. HUFF (United States) I should like to speak for myself and I believe to a certain extent for my colleagues in malaria, just a word of appreciation for Colonel Shortt's paper and to congratulate him upon his success in a very difficult undertaking. I speak with feeling because, as most of you know, Dr Coulston and I spent many man hours working on this problem after the war and were forced to close it prematurely before we had reached the fifth day of infection. We failed entirely to find any of these stages in the monkey but we failed at just the period for which Colonel Shortt also has so far failed. I should like to ask Colonel Shortt if he has yet found anything earlier than the fifth day?

Brig J. A. SIMON (United Kingdom) I am glad to have this opportunity of congratulating Professor Shortt publicly on his brilliant research work. It is noteworthy that this new light on the cycle of the mammalian malaria parasite should be announced at a time when we are commemorating the fiftieth anniversary of Ross' epoch making discovery. It is especially pleasing to me in that these two should have both been my friends and both members of the Indian Medical Service.

In the past I have always wanted some conclusive proof that an exo erythrocytic cycle occurred in mammalian malaria as in some avian infections. Professor Shortt's work leaves little doubt in the matter.

The fact that this stage of the parasite occurs in a parenchyma cell and not in one of the blood or the reticulo endothelial systems as in avian malaria, is especially interesting. What light will this shed on the differences reported in the chemotherapeutic actions of ant malarial drugs in avian as compared with mammalian hosts?

Professor Shortt has not told us of his discovery of a similar cycle in the human malaria parasite, *P. vivax*. I shall be grateful if he can give us information on this most important discovery.

Col HENRY E. SHORTT Dr Wolfson asked me whether the development of the parasite in the monkey was synchronous in the matter. The reason why we were able to get such a complete series of 5, 6, 7, 8, 9, and 10 days—was that we sometimes did these successive examinations on the same monkey. We would examine the monkey 2 days after infection—by removing a piece of liver. Two days afterwards we would remove another piece of liver, another 2 days and we would remove another piece of liver, and so on, more or less indefinitely. It didn't seem to har-

# CULTIVATION AND METABOLISM OF MALARIAL PARASITES<sup>1</sup>

QUENTIN M. GEIMAN, *Department of Comparative Pathology and  
Tropical Medicine, Harvard Medical School, Boston, Mass*

## INTRODUCTION

In the history of infectious diseases, knowledge about the causative agents has accumulated far more slowly than clinical information and the development of effective therapeutic agents. Malaria is no exception. Malaria was well described, its causative agents were known even before the discovery of the parasite, and its life cycle, and methods of transmission, interpretations of the role of the parasite in clinical relapsing, and latent malaria could be made.

Prior to the discovery of the parasites, bacteriological methods were used in attempts to isolate the agent causing malaria. Failure was the only reward for the efforts, and it was not until the work of Bass and Johns (2) in 1912 that a measure of success with cultivation was achieved. The difficulties of cultivation were soon realized when Howland (3) and Mack (4)

was obvious in the character of papers presented in 1938 before the Third International Congress.

Along with the advances in malariology, great strides were being made in biochemistry. Essential methods and techniques became available that were applicable to studies of bacterial and parasite nutrition and metabolism. The work of Christophers and Fulton (6, 7, 8), Maier and Coggeshall (9), Vohick (10), and Wendel (11) on the respiration of malarial parasites and the *in vitro* effects of antimalarial drugs started a fruitful trend in malarial research.

<sup>1</sup> The work between July 1943 and December 1945 reviewed in this paper was done in collaboration with Drs. Eric G. Ball, C. E. Anagnost, R. W. McKee and R. A. Ormsbee, Department of Biological Chemistry, Harvard Medical School, and under contract with the Committee on Medical Research of the Office of Scientific Research and Development. Since January 1, 1946, the studies reviewed have been done in collaboration with Dr. Ralph W. McKee of the Department of Biological Chemistry, Harvard Medical School and under grants in aid from the U. S. Public Health Service.

## V. MALARIA

on the whole the forms tend to be slightly larger than those of *Plasmodium cynomolgi* and on the seventh day they are approximately at the same stage of development. We have not been able, of course, to carry this experiment any further as in the case of the monkey. There is no reason to presume that the findings would be any different.

(At the end of the session Dr Shortt exhibited his specimens under microscopes in the foyer.)



## V. MALARIA

culturing the exoerythrocytic stages of *P. gallinaceum* by tissue culture procedures.

In 1943 the threat of malaria in World War II and the need for antimalarial drugs to replace the captured source of quinine called forth unprecedented fundamental research on the biochemistry of malarial parasites. In the numerous phases of an extensive program, parasitologists and biochemists were brought together in a combined effort to gain more basic information about plasmodia and indirectly to supply data of value in searching for new drugs. Evans and collaborators at the University of Chicago, Hellerman and collaborators at the Johns Hopkins University, Wendel at the University of Tennessee, and our group at Harvard University provided us with new information to further our ability to cultivate plasmodia, to understand their metabolism and pathogenesis, and to discover the mode of action of antimalarial drugs.

## IN VITRO CULTIVATION

A brief résumé of extensive studies at the Harvard Medical School (15-20) must suffice in providing the background for this account. *P. knowlesi*, developing in monkeys (*Macaca mulatta*), was used for the basic work because quantities of parasites could be produced for in vitro studies the host red cell is nonnucleated, the infection is highly pathogenic, and the organism will produce clinical malaria in man. The initial biochemical studies were handicapped by the lack of in vitro methods for growth and multiplication and prolonged observation. Consequently, experiments were devised to determine the physical and chemical environment needed by the parasites and to define the nutrients required for their in vitro growth and multiplication.

Previous studies of other workers with respiration and glycolysis were extended. The proper gas phase for incubation was sought, and analyses of monkey plasma for inorganic and organic composition were made. These studies showed that the inorganic composition of normal monkey (*M. mulatta*) serum and erythrocytes was similar to that of human beings and that glucose and amino acids were needed for respiration and metabolism of *P. knowlesi*. The conversion of glucose to lactate by the parasites at a rate 25 to 75 times that of normal red cells produced deleterious changes, both to normal red cells and to parasitized cells. This rapid and extensive consumption of glucose and the simultaneous accumulation of lactate and resulting effect on the osmotic pressure of the substrate showed that success with in vitro methods would require accurate balancing of numbers of plasmodia with concentrations of nutrients, particularly glucose.

These data from our studies and the available information on the identity and concentrations of known nutrients in monkey and human blood were used to devise a culture medium or synthetic



but our experiments did show that glucose and para aminobenzoic

the interpretation of nutrient requirements. Growth was poor and multiplication negligible in cultures using a protein free medium.

(5 times crystallized) in the synthetic medium would supply the physical properties or colloidal osmotic pressure needed by the intracellular parasite for growth and multiplication. This replacement technique whereby the parasitized blood was washed free of plasma with a modified Ringer's glucose solution and then resuspended in the medium plus 1 percent bovine albumin provided the method needed for nutritional studies. With this technique, a systematic study of the nutrition of *P. knowlesi* became possible. Studies with other species of plasmodia, *P. vivax*, *P. falciparum* and *P. cynomolgi* were also begun.

The in vitro studies are extensive, and only a small part of this work can be mentioned here. About this time, it was observed that

in the medium showed that the amount was too low and that it could be raised to 8 and 16 milligrams percent with better results. The higher concentration gave greatly improved in vitro growth and multiplication of *P. vivax*.

(23)

The application of this finding to in vivo experiments with monkeys (21) has shown that animals on fast, or on diets deficient in methionine, control their infections spontaneously. However, when methionine is present in the diet, the infection follows the normal course. In this connection, previous in vivo experiments (16) with scorbutic monkeys showed that *P. knowlesi* infections were controlled spontaneously in these animals suggesting that certain nutrients or metabolites might hold the key to levels of parasitemia or host susceptibility. The in



*vivo* interrelationship of ascorbic acid, methionine and para amino benzoic deficiencies on the course of *P. knowlesi* infection have been studied, but they are not completed (23). The data from *in vivo* and *in vitro* studies show that ascorbic acid is indirectly essential to the metabolism of *P. knowlesi* *in vivo* but not *in vitro*, and that methionine is directly essential both *in vivo* and *in vitro* for the growth and multiplication of *P. knowlesi*. That these results are important in helping to explain the host parasite relationship controlling levels of parasitemia and course of infection is obvious.

The above *in vitro* techniques have been used to compare the specific nutrition and metabolism of other species of plasmodia. *P. vivax* requires a greater amount and *P. falciparum* a lesser amount of amino acids than *P. knowlesi* for *in vitro* growth (23). Even though *P.*

and actual destruction of the host red cell by the growth of *P. cynomolgi*, explain the lower metabolic rate of this parasite is not known. *P. falciparum* is similar biochemically to *P. knowlesi* in many ways, and the two parasites cause minimal damage to host cells during normal growth. We are hoping to explain the biochemical affinities of *P. falciparum* for the small capillaries and the ability of this organism to multiply so rapidly in the blood stream during primary infection. From our studies with factors limiting levels of parasitemia in *P. knowlesi* infections, key metabolites for the growth and multiplication of *P. falciparum* in man apparently exist in the blood stream in adequate amounts, or the parasite can synthesize needed nutrients from circulating substances available in plasma.

TABLE 1 — Comparative rates of glycolysis and respiration for 4 species of malarial parasites<sup>1</sup>

| Species of parasites | Utilization in mM/hr/1x10 <sup>8</sup> parasites |         |                      |         | Oxygen uptake in mM <sup>2</sup> /hr/1x10 <sup>8</sup> parasites |         |
|----------------------|--|---------|----------------------|---------|--|---------|
|                      | Glucose  |         | Lactate <sup>2</sup> |         | Range  | Average |
|                      | Range  | Average | Range                | Average |  |         |
| <i>P. knowlesi</i>   | 3-9  | 5       | 3-9                  | 5       | 8-25   | 14      |
| <i>P. cynomolgi</i>  | 3-5  | 4       | 3-6                  | 4       | 7-17   | 10      |
| <i>P. falciparum</i> | 6-8  | 7       | 6-8                  | 7       |  |         |
| <i>P. vivax</i>      | 15-50  | 20      | 15-50                | 20      |  |         |

<sup>1</sup> Age of parasite determines rate of glycolysis and respiration. Old parasites have approximately 3 times the activity of young stages.

<sup>2</sup> 1 mM glucose forms 2 mM lactate causing accumulation and acidity of substrate in culture.

The ability to obtain *in vitro* growth and multiplication of plasmodia by methods described above, suggested early in our studies that the methods might be useful in studying drug action uncomplicated

but our experiments did show that glucose and para aminobenzoic acid were essential for growth and that the blocks of substances enhanced growth and multiplication in vitro. Further fractionation of the blocks was not possible because of the presence of plasma in the mixtures being cultivated. The masking effects of the plasma nullified the interpretation of nutrient requirements. Growth was poor and

(5 times crystallized) in the synthetic medium would supply the physical properties or colloidal osmotic pressure needed by the intracellular parasite for growth and multiplication. This replacement technique whereby the parasitized blood was washed free of plasma with a modified Ringer's glucose solution and then resuspended in the medium plus 1 percent bovine albumin provided the method needed for nutritional studies. With this technique, a systematic study of the nutrition of *P. knowlesi* became possible. Studies with other species of plasmodia, *P. vivax*, *P. falciparum* and *P. cynomolgi* were also begun.

The in vitro studies are extensive, and only a small part of this work can be mentioned here. About this time, it was observed that

cation. This was interpreted as a lack of building blocks in the medium for protein synthesis. Titration of amino-acid concentration

tiplication of *P. vivax*

Experiments with a mixture of synthetic essential and nonessential amino acids gave results approximately the same as those obtained

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Table 2.—*In vitro effects of drugs on P. cynomolgi*<sup>1</sup>

| Parasite mix                    | pH<br>10 hr | Glucose<br>utiliza-<br>tion<br>10 hr | Lactate<br>utiliza-<br>tion<br>10 hr | Para-<br>site<br>counts<br>0/10 hr | Parasite differential |       |     |      |      |                         |         | Parasite evaluation |                      |         |         | Increase<br>total |
|---------------------------------|-------------|--------------------------------------|--------------------------------------|------------------------------------|-----------------------|-------|-----|------|------|-------------------------|---------|---------------------|----------------------|---------|---------|-------------------|
|                                 |             |                                      |                                      |                                    | Ring                  | Troph |     | Sch. | Seg. | Degen-<br>and<br>X-cell | Normal  | Identifi-<br>fiable | Noniden-<br>tifiable | Free    |         |                   |
|                                 |             |                                      |                                      |                                    |                       | Young | Old |      |      |                         |         |                     |                      | Percent |         |                   |
|                                 |             |                                      |                                      |                                    |                       |       |     |      |      |                         |         |                     |                      | Percent | Percent |                   |
| BM                              | --          | 7.31                                 | Mg/Pd                                | Percent                            | 3                     | 84    | 20  | 3    |      | 2                       | Percent | Percent             | Percent              | Percent | Percent |                   |
| BM atabrine 1 000 gamma/l       | 7.31        | 156                                  | 91                                   | 1.9                                | 5                     | 85    | 1   | 3    |      | 3                       | 56      | 11                  | --                   | 3       | 12.9    |                   |
| BM chloroquine 1 812 gamma/l    | 7.52        | 113                                  | 14                                   | 6.6                                | 15                    | 81    | 1   | 3    |      | 3                       | Rare    | 7.5                 | 20                   | 20      | 2.2     |                   |
| BM pentamidine 1 250 gamma/l    | 7.51        | 106                                  | 23                                   | 2                                  | 14                    | 46    | 2   | 20   | 2    | 16                      | 3       | 82                  | 3                    | 14      | 2.3     |                   |
| BM isopentamidine 1 550 gamma/l | 7.49        | 162                                  | 72                                   | 4.4                                | 9                     | 8     | 1   | 3    |      | 9                       | 10      | 81                  | 1                    | 8       | 12.3    |                   |
| BM paludrine 3 000 gamma/l      | 7.46        | 169                                  | 67                                   | 2.9                                | 7                     | 87    | 1   | 4    |      | 2                       | 18      | 80                  | 1                    | 1       | 21.5    |                   |
|                                 | 7.33        | 167                                  | 27                                   | 2.6                                | 7                     | 73    | 2   | 12   |      | 5                       | 17      | 77                  | 2                    | 4       | 23.0    |                   |

(Rockley differentials on bottom left and right)

<sup>1</sup> Rocker dilution cultures M 215 in a synthetic medium (21) Morphological evaluation of the plasmodia at end of the culture arbitrarily classifies all parasites as normal, identifiable, nonidentifiable, or free of red cells.

by host factors. Quinine, quinacrine, and sulfadiazine were chosen for initial studies (25), but more recently numerous experiments have been performed with the newer antimalarial drugs (21) including chloroquine (SN-7618), SN-13276 (pentaquine), SN-13274 and paludrine (SN-12837). Comparative *in vitro* studies have been made on the same generation of parasites being cultivated in the presence of plasma and with plasma free media. Earlier studies showed that quinine and quinacrine had a direct and prompt action

that the parasites require para aminobenzoic acid for growth. In fact, the *in vitro* effects of sulfadiazine can be completely antagonized by para aminobenzoic acid. Similar *in vivo* results have been obtained by Marshall (26) for *Plasmodium lophurae* and by Richardson et al (27) for *P. knowlesi*.

The type of experiment shown in table 2 demonstrates the action

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direct addition to cultures, acts more slowly. Subcultures have to be used to detect the effects of therapeutic levels. Thus this drug appears to act similarly to sulfadiazine by interfering with growth. The inhibition of nuclear division as reported by Fairley (28) and Black (29) — — — — —

effects of the drug with those exerted by its degradation products

### METABOLISM

Interpretation of the results of the following studies

female anopheline mosquitoes are responsible for infection when the insect bites a new host, the stages resulting from invasion of erythrocytes are the prime cause of the morbidity and mortality caused by malaria. No metabolic information is available as yet about the newly discovered tissue stages or cryptozites of avian and mammalian plasmodia, but the information about the metabolism of asexual stages has increased greatly since the studies of Christophers and Fulton (6-8). Metabolic studies have been carried out in various

Table 2.—*In vitro* effects of drugs on *P. cynomolgi*<sup>1</sup>

| Parasite mix                    | pH<br>19 hr | Glucose<br>utiliza-<br>tion<br>19 hr | Lactate<br>utiliza-<br>tion<br>19 hr | Para<br>site<br>counts<br>Q/10 hr | Parasita differential |       |     |     |     |                         | Parasita evaluation |                     |                      |      | Increase<br>total |      |
|---------------------------------|-------------|--------------------------------------|--------------------------------------|-----------------------------------|-----------------------|-------|-----|-----|-----|-------------------------|---------------------|---------------------|----------------------|------|-------------------|------|
|                                 |             |                                      |                                      |                                   | Ring                  | Troph |     | Sch | Seg | Degen-<br>and<br>X-cell | Normal              | Identifi-<br>fiable | Noniden-<br>tifiable | Free |                   |      |
|                                 |             |                                      |                                      |                                   |                       | Young | Old |     |     |                         |                     |                     |                      |      |                   |      |
|                                 |             |                                      |                                      |                                   |                       |       |     |     |     |                         |                     |                     |                      |      |                   |      |
| SM                              | 7.31        | 156                                  | Mg/Pd                                | Mg/Pd                             | Percent               | 9     | 54  | 35  | 3   | 3                       | 3                   | 66                  | 11                   | 3    | 3                 | 22.9 |
| SM salarins 1 000 gamma/l       | 7.31        | 156                                  | 61                                   | 1.0                               | 9                     | 66    | 1   | 3   | 3   | 3                       | 3                   | None                | 8                    | 20   | 20                | 2.2  |
| SM chloroquine 1 612 gamma/l    | 7.52        | 112                                  | 34                                   | 5.6                               | 9                     | 13    | 61  | 2   | 2   | 2                       | 22                  | None                | 8                    | 16   | 16                | 2.2  |
| SM pentamidine 1 250 gamma/l    | 7.51        | 108                                  | 33                                   | 3                                 | 13                    | 14    | 46  | 2   | 20  | 2                       | 16                  | 3                   | 83                   | 3    | 3                 | 2.2  |
| SM isopentamidine 1 250 gamma/l | 7.46        | 162                                  | 72                                   | 4.4                               | 9                     | 78    | 1   | 1   | 1   | 1                       | 9                   | 10                  | 81                   | 1    | 1                 | 2.2  |
| SM paludrine 3 000 gamma/l      | 7.33        | 160                                  | 67                                   | 2.9                               | 7                     | 97    | 2   | 12  | 3   | 9                       | 19                  | 80                  | 1                    | 1    | 1                 | 2.2  |
|                                 |             | 167                                  | 37                                   | 3.8                               | 7                     | 73    | 2   | 12  | 3   | 9                       | 17                  | 77                  | 1                    | 1    | 1                 | 2.2  |

3 Becker diffusion medium No. 201

<sup>1</sup> Rocker diffusion culture N 225 in a synthetic medium (21). Morphological evaluation of the plasmodia at end of the culture arbitrarily classifies all parasites as normal, identifiable, nonidentifiable, or free of red cells.

laboratories with three species of simian parasites, *Plasmodium* *leesii*, *P. inui* and *P. cynomolgi*, three species of avian parasites, *Plasmodium cathemerium*, *P. lophurae*, and *P. gallinaceum* and two species of human plasmodia, *P. vivax* and *P. falciparum*.

The majority of the studies have been concerned with carbohydrate metabolism and its inhibition by antimalarial drugs, but certain aspects of protein and lipid metabolism have been investigated. Nevertheless, the studies and results thus far appear to follow a general pattern illustrated in the following diagram (fig 2).

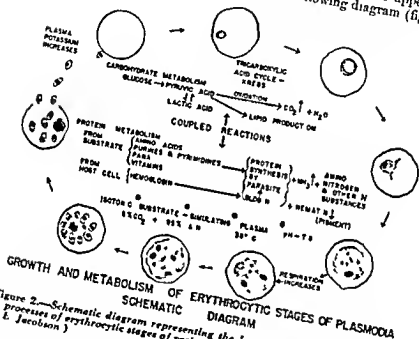


Figure 2.—Schematic diagram representing the known growth and metabolic processes of erythrocytic stages of various species of plasmodia (Drawn by E. Jacobson)

considering the diagram, the sequence of events in the growth, multiplication and morphological changes of the plasmodia must be visualized. The morphological changes are progressive and the chemical and metabolic processes are coupled. Various types of suspensions have been used for biochemical and metabolic studies of plasmodia. Suspensions of intact parasitized and suspensions of free parasites obtained by laking host cells with hemolytic agents such as saponin, distilled water, or red-cell lysis have been used (17, 29, 30, 31). The development of in vitro cultural methods which would permit prolonged growth and

Table 2.—*In vitro* effects of drugs on *P. cynomolgi*†

| Parasite mix                    | pH<br>10 hr | Glucose<br>utiliza-<br>tion<br>10 hr | Lactate<br>utiliza-<br>tion<br>10 hr | Para-<br>site<br>counts<br>0/10 hr | Parasite differential |       |     |     |     |                         | Parasite evaluation |                     |                      |         | Increase<br>total |      |
|---------------------------------|-------------|--------------------------------------|--------------------------------------|------------------------------------|-----------------------|-------|-----|-----|-----|-------------------------|---------------------|---------------------|----------------------|---------|-------------------|------|
|                                 |             |                                      |                                      |                                    | Ring                  | Troph |     | Sch | Seg | Degen.<br>and<br>X-cell | Normal              | Identifi-<br>fiable | Noniden-<br>tifiable | Free    |                   |      |
|                                 |             |                                      |                                      |                                    |                       | Young | Old |     |     |                         |                     |                     |                      |         |                   |      |
|                                 |             |                                      |                                      |                                    |                       |       |     |     |     |                         |                     |                     |                      |         |                   |      |
| SM                              |             |                                      |                                      |                                    | 3                     | 54    | 20  | 3   |     | 2                       | Percent             | Percent             | Percent              | Percent | Percent           |      |
| SM                              | 7.31        | 156                                  | Me/Fet                               | Percent                            | 3                     | 55    | 1   | 3   |     | 3                       |                     | 30                  | 11                   | 2       | 3                 | 12.9 |
| SM chloroquine 1 000 gamma/l    | 7.52        | 112                                  | 81                                   | 3.6                                | 15                    | 51    | 3   | 3   |     | 22                      |                     | 30                  | 78                   | 2       | 20                | 2.2  |
| SM pentamidine 1 000 gamma/l    | 7.61        | 106                                  | 24                                   | 3                                  | 14                    | 49    | 2   | 20  | 2   | 16                      |                     | 2                   | 83                   | 2       | 14                | 2.2  |
| SM isopentamidine 1 250 gamma/l | 7.46        | 162                                  | 33                                   | 3                                  | 9                     | 75    | 1   | 3   |     | 3                       |                     | 10                  | 81                   | 2       | 8                 | 22.3 |
| SM paldurine 1 000 gamma/l      | 7.48        | 160                                  | 67                                   | 2.9                                | 7                     | 67    | 3   | 4   |     | 6                       |                     | 18                  | 80                   | 1       | 1                 | 21.5 |
|                                 | 7.53        | 167                                  | 37                                   | 2.8                                | 7                     | 73    | 2   | 12  |     |                         |                     | 27                  | 77                   | 2       | 5                 | 22.0 |

! Rocker dilution culture M 225 in a synthetic medium (21) Morphological evaluation of the cultures  
identifiable, nonidentifiable, or free of red cells

† Röcker dilution culture M 225 in a synthetic medium (21). Morphological evaluation of the plasmodia at end of the culture arbitrarily classifies all parasites as normal, identifiable, nonidentifiable or free of red cells.

laboratories with three species of simian parasites, *Plasmodium knowlesi*, *P. inui* and *P. cynomolgi*, three species of avian parasites, *P. cathemerium*, *P. lophurae*, and *P. gallinaceum* and two species of human plasmodia, *P. vivax* and *P. falciparum*.

The majority of the studies have been concerned with carbohydrate metabolism and its inhibition by antimalarial drugs, but certain aspects of protein and lipid metabolism have been investigated. Details of the chemical and metabolic studies cannot be presented at this time. Nevertheless, the studies and results thus far appear to follow a general pattern illustrated in the following diagram (fig 2)

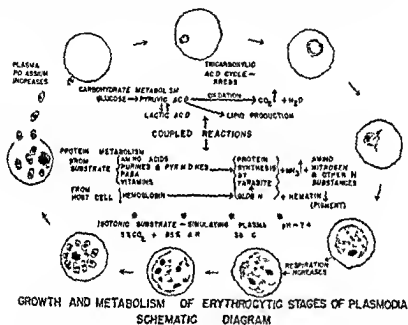


Figure 2.—Schematic diagram representing the known growth and metabolic processes of erythrocytic stages of various species of plasmodia (Drawn by L. Jacobson)

In considering the diagram, the sequence of events in the growth, the plasmodia must progressive and the

Various types of suspensions have been used for biochemical and metabolic studies of plasmodia. Suspensions of intact parasitized cells and suspensions of free parasites obtained by laking host cells with hemolytic agents such as saponin, distilled water, or red-cell antiserum have been used (17, 20, 30, 31). The development of in vitro cultural methods which would permit prolonged growth and



multiplication through successive generations provided new tools to study the metabolism of parasites under controlled conditions and also to determine the utilization of nutrients by use of chemical and bio assay methods

Glucose metabolism is highly essential for the life of the malarial parasite. Comparisons show that *P. gallinaceum* utilizes about 70 times (31), and *P. knowlesi* 25 to 75 times (17) more glucose than the normal red cell. Thus glucose is utilized to form lactic acid a part of which is subsequently oxidized to carbon dioxide and water. The formation of two molecules of lactic acid from each molecule of glucose and the partial utilization of the lactate leads to a rapid accumulation of acid which conditions the life of cultures. The intermediate metabolism of glucose through pyruvate appears to act

pounds are accessory rather than essential nutrients

In considering the protein metabolism of malarial parasites two sources of protein are available for the relatively rapid growth or synthesis of protoplasm by the malarial parasite. The hemoglobin of the host cell is utilized and amino acids and possibly peptides diffuse through the cell membrane for synthesis by the parasite into protein. Hemoglobin metabolism by malarial parasites has been (20), and there is agreement hematin and globin by the pigment, and the globin is metabolized as a source of amino acids. From the in vitro cultural

acids (25) during the growth of the parasites must also be derived largely from the substrate. Moulder and Evans (33) demonstrated

nitrogenous materials will be of value in detecting the presence of malarial toxins.

Very little is known about lipid metabolism of plasmodia. Analyses of parasite substance (25) show a great increase of lipid in the

laboratories with three species of simian parasites, *Plasmodium knowlesi*, *P. inui* and *P. cynomolgi*, three species of avian parasites, *P. relictum*, *P. lophurae*, and *P. gallinaceum* and two species of

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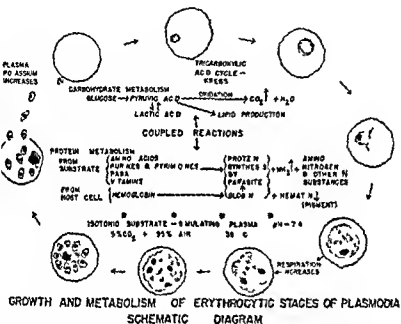


Figure 2—Schematic diagram representing the known growth and metabolic processes of erythrocytic stages of various species of plasmodia (Drawn by E. Jacobson)

In considering the diagram, the sequence of events in the growth, segmentation reinvasion and multiplication of the plasmodia must be visualized. The morphological changes are progressive and the biochemical and metabolic processes are coupled.

Various types of suspensions have been used for biochemical and metabolic studies of plasmodia. Suspensions of intact parasitized cells and suspensions of free parasites obtained by laking host cells with hemolytic agents such as saponin, distilled water, or red-cell antiserum have been used (17, 29, 30, 31). The development of in vitro cultural methods which would permit prolonged growth and

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cytoplasm of *P. knowlesi*, and quantitatively this parasite contains no weight. It appears of relatively great

## CONCLUSION

An analysis of the cultural and metabolic data shows that the cultivation of blood stages of plasmodia requires precision technique and that the host cell alone cannot supply the essential nutrients for growth and multiplication. Plasmodia behave like other living cells in their basic biochemical and metabolic requirements. The enzyme systems of *Plasmodium gallinaceum* and *P. knowlesi* that have been studied are analogous to those of other tissue cells. Nevertheless, the mechanism of pigment production from hemoglobin, the invasion of the red cell and dependence of the parasites on the intracellular environment, and the differential sensitivity between plasmodia and tissue cells for antimalarial drugs suggest the existence of differing enzyme systems and pathways of metabolism.

Furthermore, the availability in the substrate of at least several essential nutrients such as glucose, para-aminobenzoic acid and amino acids determines the amount of growth and multiplication of the parasites.

The need for diffusible nutrients from the plasma is a clue to the existence of plasma and hence host factors which control levels of parasitemia and pathogenicity. Unlimited prolonged cultivation of mammalian plasmodia awaits better methods for the maintenance of the integrity of erythrocytes *in vitro*, the elaboration of specific biochemical and metabolic properties of plasmodia, the identification of unknown growth promoting substances in plasma, or the ability to provide an intracellular medium which will permit growth and multiplication free of the red cell.

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ments with the Madagascar strain, in which "recrudescence" indicated a renewal of activity within 8 weeks of recovery from the primary attack, "relapse" a renewal within 8 to 24 weeks, and "recurrence" a renewal later than 24 weeks, is not practicable for general use nor is it relevant for all strains of *P. vivax*. We agree that the term "recrudescence" has a certain usefulness, but we employ it only for that type of relapse which can best be explained by the survival of erythrocytic parasites without the necessity of new parasites entering the circulation from a fixed tissue reservoir. We do not feel that it is always possible to distinguish recrudescence, as so defined, from true relapse, because either may follow primary or secondary episodes of parasitemia, and the time elapsing between treatment and renewal of activity is not an unfailing criterion.

During the past 6 years we have studied, under controlled conditions, over 300 subjects experimentally infected with *P. vivax*, most of them were observed for periods of 18 months or more after infection. Analysis of the relapses in these subjects has led us to several conclusions, some definite and others tentative, as to the influence of the strain of parasite, dosage of sporozoites, acquired immunity, and specific therapy upon the occurrence and spacing of relapses. In the ensuing presentation emphasis is placed upon the influence of the strain of parasite, because of the limitations of space and because the evidence supporting its relative importance is most complete.

#### INFLUENCE OF STRAIN OF PARASITE

A striking characteristic of *vivax* malaria is its tendency to delayed primary attacks and delayed relapses occurring 6 to 12 months after infection. One of the first experiments aimed at proving the "mosquito theory" of malaria transmission illustrates this phenomenon. In 1900 Sir Patrick Manson, carrying out transmission studies in what he termed a "dramatic and crucial manner," arranged to have *vivax* infected mosquitoes brought from Italy to London. One of several volunteers bitten by these mosquitoes was P. Thurburn Manson, Sir Patrick's 23 year old son. The younger Manson developed primary malaria after about 2 weeks and was given quinine. He remained in normal health until 9 months later when, while in Scotland he had a typical relapse which he himself reported in some detail (Manson, 1901). Another volunteer in the same pioneer period a Major Fearnside, reported (1903) a similar personal experience.

These early isolated reports of 8 and 9 month intervals between

of *vivax* malaria in many countries. Hackett (1934), Gilchrist (1934), Kikuth (1943), and Shute (1946), as well as others, emphasize the importance of this characteristic. Hackett pointed out how it provided an explanation both for spring malaria and for the overwinter

## RECRUDESCENCE AND RELAPSE IN VIVAX MALARIA

G ROBERT COATNEY and W CLARK COOPER, *Subdivision on Division of Tropical Diseases, National Institute of Bethesda, Md*

In 1897, almost at the time when Ronald Ross was completing his basic studies on the transmission of malaria, Thayer (1897) published a series of lectures which contain several illuminating references to relapse in vivax malaria. He distinguished "recrudescences" from relapse, followed "imperfect and insufficient treatment" from later renewal activity which followed adequate treatment and apparently complete recovery. In his speculations as to the explanation for late relapse Thayer agreed with the suggestions of earlier investigators that there must exist some undiscovered form of the parasite and he wrote "the organism may remain perhaps within the cell body of certain phagocytes for long periods of time, only to be set free again as a result of some insult, the nature of which is not as yet appreciable to us. Fifty years later, we still do not know where the persistent form of *Plasmodium vivax* remain or the mechanism which initiates renewal of activity. Recent advances in the histopathology of the avian and simian malarial (Huff, 1917, Shortt, Garnham, and Malamos, 1949), however, make one feel that the first of these basic deficiencies may any day be removed. Despite lack of direct proof, indirect evidence is strong enough for general acceptance of the hypothesis that fixed tissue forms of *P. vivax* are responsible for the maintenance of the infection and that these periodically release parasites which can invade red blood cells. The evidence for such an hypothesis has recently been reviewed by Darey (1946), Huff (1947), and Coatney and Cooper (1948) and will not be dwelt upon here. In stead, the major emphasis will be upon patterns of relapse in vivax malaria and upon some of the factors which influence the incidence and spacing of these relapses. Saperro (1947) has recently discussed some of the implications of such variables in an excellent review of current concepts of relapsing malaria.

In the present state of our knowledge, renewal of parasitic activity in vivax malaria must refer to erythrocytic parasitemia, that is, to the reappearance of, or the rapid increase in the number of, circulating erythrocytic parasites after a period of quiescence, reinfection being excluded. We prefer to use the term "parasitic relapse" in the broad sense to include all reactivations irrespective of the time in relation to exposure and treatment, regardless of whether the relapse is the result of surviving erythrocytic parasites or the result of reinvasion of the blood from an exo-erythrocytic reservoir. In this, we are in agreement with the League of Nations Subcommittee (1940). The terminology introduced by James (1931) to meet his special require-



ing of the parasite and suggested that strains of this type a greatly enhanced chance of survival in the temperate zone.

Evidence that this consistently long interval between primary and secondary activity is common has come from many painstaking epidemiological studies of Korteweg (1921) in the Netherlands, also at once to mind in this connection. It has been also upon observations in individuals who have moved from malarious to nonmalarious areas, as, for example, in reports such as of Martini (1934), Wilckens (1943), Horing (1946), and He (1947). But the most convincing evidence has come from studies of experimentally infected subjects where delayed activity, characteristic of season. Thus, Warrington Yorke (1924), who pioneered the use of mosquito-transmitted malaria in paretics, commented on late relapses 6 to 13 months after exposure in the first group of patients in whom he induced *P. vivax*. James and his associates (1931, 1936) gave abundant proof of similar characteristics in the confirmed in experimentally infected volunteers the prolonged latency of indigenous Dutch strains which Korteweg (1921) had earlier deduced from morbidity data. In the United States, Boyd and Kitchen (1914) produced evidence that the McCoy strain and other American strains of *P. vivax* have a bimodal activity pattern. Shannon et al. (1948) likewise described late relapses of McCoy strain vivax malaria.

If the development of vivax strains with a tendency to delayed relapse has been through selective survival in areas with short transmission seasons (Hackett, 1937), then one would not expect such strains to be common in areas where transmission extends over a great portion of the year. Despite frequent allusions in the literature to the likelihood that not all vivax strains are alike in their relapse patterns, until recently no direct comparisons have been available. With the war in the Pacific, between 1941 and 1945, however, morbidity statistics were obtained which showed clearly that the vivax malaria acquired in the Solomon Islands, New Guinea, and other Southwest Pacific areas did not exhibit a uniform pattern of prolonged latency. Corroborating this, Fairley (1945), working in Australia with strains of *P. vivax* imported from New Guinea, likewise reported no patterns of delayed relapse. The question remained, however, whether or not the apparent differences in relapse activity reflected true strain differences or whether they might be explained by differences in the technique or intensity of exposure, or by the different environments in which the individual infections were acquired and developed.

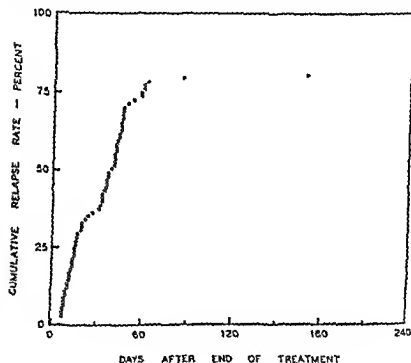
Since 1942 we have utilized two different strains of *P. vivax* for the drug tests in prisoner volunteers, both strains were studied under similar conditions. One of these, the St. Elizabeth, was isolated in the United States, but it is not known how long it has been



were interrupted by treatment, activity came to an end after about 14 or 15 months

Other observations with this strain have included the following

of late erythrocytic parasitemia; (4) allowing late attacks to go untreated results in several months of remittent and intermittent patent parasitemia, the final termination of which is often later than in in



dividuals in whom each late attack is treated; and (5) the infection can be cured with pentaquine, an 8 aminoquinoline derivative, given with quinine either during the early attack, during latency or during the first late attack.

The activity pattern of the St Elizabeth strain can best be explained by postulating a relatively short period between 7 and 14 days after exposure during which red cell invading parasites enter the circulation, then a long period of many months when the fixed tissue

During the early weeks after exposure, overt activity can react suppressed by brief administration of a drug capable of interrupting the erythrocytic cycle and this suppression is followed by many more attacks. Therapy during the early attack is followed by a comparable period of latency. When one plots the time intervals of treatment to first relapse after 72 early primary attacks, the pattern is plainly evident (fig 2). The 15 early relapses all follow treatment with deliberately low dosages of quinine or with NIH a relatively poor schizonticide (Cooper and Costney, 1947), and interpreted as recrudescences because we do not believe that the erythrocytic parasites were eliminated. When full courses of quinine

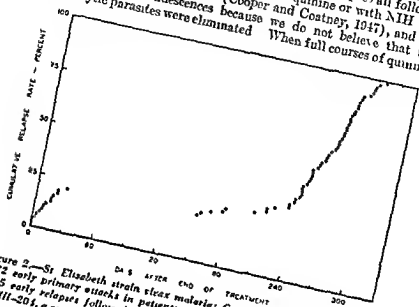


Figure 2.—St. Elisabeth strain *Stear malaris*: Cumulative relapse rates after 72 early primary attacks in patients treated with noncurative drugs. The 15 early relapses followed treatment with very small doses of quinine or NIH-201, a comparatively poor antimalarial.

quinacrine, chloroquine, and other effective antimalarials were used, however, prolonged latency invariably resulted, late relapses appearing 172 to 302 days after therapy, or 179 to 327 days after infection. The course of events following treatment of late attacks was quite different. Here, as shown in figure 3 prompt relapses usually occurred after full courses of quinine, quinacrine, chloroquine or other noncurative drugs. This was true whether the initial late attack was primary or relapse. During the period of maximum relapse activity, 270 to 330 days after exposure, the treatment to relapse intervals were roughly proportional to the persistence of effective concentrations of the respective drugs in the human host. In subjects in whom all late attacks

excessive alcohol intake, injections of epinephrine, etc. will make latent malaria become overt, there is a dearth of controlled evidence. Bianco et al (1947) were unable to induce relapses by a variety of such means. In the planning and appraising of experiments bearing upon this problem, a distinction should be made between (1) relapses within a few hours after the insult, which by necessity would have to be explained by a redistribution of already existing erythrocytic parasites, and (2) relapses at longer intervals after the stimulus, which could be explained by the disturbance of an immune barrier against subpatent erythrocytic infection or by emergence of parasites from an exo erythrocytic site.

### SUMMARY AND CONCLUSIONS

In the study of over 300 experimental sporozoite induced vivax infections we found that two strains of *P. vivax*, the St Elizabeth and the Chesson, have strikingly different relapse patterns. We also obtained evidence, of varying degrees of conclusiveness, that the dosage of sporozoites, the acquisition of acquired immunity and the nature of the drug used in therapy significantly affect the spacing and the probability of relapses. The importance of these variables, even within the confines of a standardized experiment, helps to explain the bewildering complexity of group relapse characteristics of natural malaria and illustrate why alternate case controls and prolonged periods of observations are necessary in all comparative studies of drugs and relapse rates.

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# V MALARIA

parasites remain quiescent, followed by a late period during which there are repeated invasions of the erythrocytes.

We have so far observed 152 volunteers infected with the Chesson strain of *P. vivax*, which, it will be recalled, was of Southwest Pacific origin. Each of these subjects was bitten by 10 infected mosquitoes. While only 56 of these men have so far been observed for a full year, there is ample evidence that the activity pattern is radically different from that of the St. Elizabeth strain. This confirms the pilot study quoted in a footnote by Gordon et al. (1947) and is in agreement with the findings of the Chicago group (Whorton et al. 1947) and of Shannon et al. (1948).

The frequency distribution, by week of onset, of 280 attacks of Chesson strain *vivax* malaria is shown in figure 4. There is no tendency toward early and late periods of activity. In subjects given

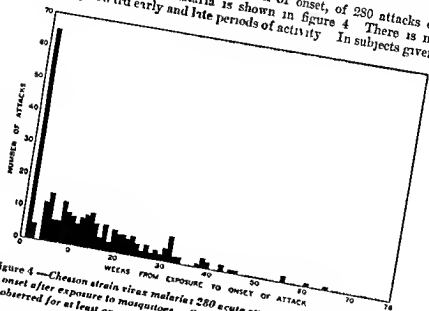


Figure 4.—Chesson strain *vivax* malaria: 280 acute attacks arranged by time of onset after exposure to mosquitoes. Only attacks in subjects who have been observed for at least one year are included.

protective drugs patent parasitemia appeared soon after the drug had been removed from the host whether medication was stopped 6 days after exposure or was continued for a year. When a Chesson attack was interrupted by full therapy, relapse usually occurred promptly, the cumulative relapse rate following therapy of primary attacks reached 3. Recurrent activity of infections has continued for more than 18 months in some individuals. In men who received comparatively weak inocula of Chesson sporozoites there was no tendency to a St. Elizabeth strain

# AUTOCHTHONOUS MALARIA IN AUSTRIA

H. M. JETTMAR, *Institute of Hygiene, Graz*

Outbreaks and isolated cases of autochthonous malaria seem to have occurred long ago in the territory of present Austria. It is known, for instance, that in the Danube marshes and in the Marchfeld plain east of Vienna there were severe outbreaks of malaria in the middle of the last century (6). In the southern part of Styria a big pond serving for pisciculture had to be drained in 1880 in connection with the appearance of febrile diseases which occurred among the local population (5).

In the first 15 years of the twentieth century only isolated cases were reported and malaria seemed to be a rare disease here, but it should be mentioned that malaria has been classed as a notifiable disease only since 1924.

After the first World War there was a sudden rise of cases among the Viennese civilian population in connection with the return of numerous soldiers and prisoners of war from the Balkan and Eastern fronts. Thus 3 717 cases were registered in Vienna in 1919.

Most of these cases were, of course, not autochthonous but were introduced from endemic malaria foci south and east of Austria. Although local anopheline mosquitoes had been infected and had undoubtedly produced some new autochthonous cases, the malaria subsided rapidly during the following years, when the incidence of this disease was reduced again to a few sporadic cases per annum.

## THE THREE FOCI IN SOUTHERN AND EASTERN STYRIA

County where, altogether, 97 cases were registered. Most of these cases occurred during the years 1937-39. Antiepidemic measures were taken on a larger scale (drainage of some ponds, inspection of houses, and treatment of all patients). Thus the outbreak subsided rapidly. Since 1940, only sporadic cases have been reported, and after 1943 no more cases were reported.

The basin of Arnfels has a very warm climate and is meteorologically regarded as a xerothermic island (5). It is about 300 meters above sea level.

*Kaiserwald, south of Graz*—On the other hand, two new foci became active after 1940 near Graz. A small one in the valley of the river Raab near Kirehbach, and a bigger one quite near to the capital of Styria around the Kaiserwald (Emperor's Forest) a distance of



and Viennese Forest) but also in the suburbs inside of the Vienna city district. *A. bifurcatus* and *A. nigripes* are encountered here as well although not in such an abundant quantity (3)

*Environs of St Poelten*—Another, but smaller, outbreak of tertian malaria occurred in the same year independently in the western part of Lower Austria near St Poelten, in some villages. Altogether 16 cases, all of local infection, were admitted and investigated in the town hospital of St Poelten (about 40 miles west of Vienna). The villages from which the cases were reported are situated along the valley of the river Traisen at an altitude between 200 and 300 meters above sea level.

#### UPPER AUSTRIA

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| <p><i>Near Gmunden city</i>—In 1916<br/> <i>Plasmodium vivax</i>, was observed<br/>         near Gmunden city, 34<br/>         The disease arose from a local source on the northern slope of the town<br/>         where numerous wounded and sick soldiers were lodged. Most of<br/>         them had come from the</p> | <p><i>Plasmodium</i><br/>         district<br/>         ear (4)</p> |
|---|---|

The formation of new endemic foci of tertian malaria in Austria during the last few years has been caused partly by immigration of numerous carriers of gametocytes of *Plasmodium vivax*, and is partly due to the uncommon meteorological conditions during the last summers, especially during the spring and summer of 1916. The extraordinarily high temperature during the three last summers (1915-17) has been very favorable for the development of the sexual phase of the *Plasmodium vivax* in the local *Anopheles maculipennis* (messeae and/or *typicus*)

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This fact gives us reason to hope that the mentioned and at present still active foci of malaria will again gradually subside when the climatic conditions return to normal and all patients are brought under control.

On the other hand, it has to be taken into consideration that very protracted incubation periods are possible and that patients may escape control and treatment.

only about 10 miles south of Graz. Here, as well as in the mentioned above, *Anopheles maculipennis* var *messeae* and *A. lipennis* var *typicus* are the vectors. This species breeds in numbers in the numerous ponds, marshes, and other stagnant collections around the big forest. The forest itself is almost habited and is situated on extended and low hills (350 m a s), around it on the slopes and in the valleys of the Mur and Kai rivers (altitude 300 m a. s.) there are located numerous villages. Altogether, 74 cases of certain autochthonous malaria (all tertiary) were registered here during 1945-47, while 17 cases were introduced. The reason why malaria invaded the local population to such a comparatively high scale (97 among 5,700 population=1.7 per cent) was the migration during wartime and afterward. A camp of foreign laborers had been established there in 1941, and the district was used repeatedly by troops and refugees for camping.

*Anopheles maculipennis* hibernates here in the cellars used by peasants for storage of vegetables. It is not found in stables or in the woodwork under the roofs during the cold season. The anophelines rest in the darkest parts of the cellars, on the higher places of the walls, and on the ceilings, associated with a sometimes immense number of hibernating culicines. Provided the local population guarantees full collaboration, an effective campaign against hibernating anopheline mosquitoes is, therefore, possible.

**Airdbach a d Raab, Eastern Styria.**—The second new focus of malaria is situated in Southeastern Styria in the Raab Valley near Kirchbichl at a distance of about 20 miles from Graz. Only 23 cases occurred there. Anophelism is supported here mostly by ponds with marshy shores. One of these ponds, from where this outbreak has been supposed to have started (5), is used for pisciculture. The valley is broad and only about 300 meters above sea level. The climate is mild, and the summers are hot. During the years 1945-47 the springs and summers were especially hot and dry. The focus was established in 1945, evidently by infected troops and refugees.

#### LOWER AUSTRIA

**Outbreaks near Vienna.**—Further outbreaks of malaria occurred recently in Lower Austria in the southern and southeastern environs of the Austrian capital. They originated chiefly from returning soldiers and prisoners of war with numerous carriers of tertian gametocytes among them (6). In 1946 140 cases, among them 63 certain autochthonous ones, were registered inside Vienna city. Anophelism in and around Vienna is very common. *A. maculipennis* met with not only in the environs (Viennese Basin, Danube marshes,

Inside of the territory of the city of Graz this species is met with in very great numbers, and some ponds belonging to the municipality and being kept partly for the fire-brigade run with larvae of *A. maculipennis*, while *A. messeae* breeds in some brooks in the vicinity.



## Session 2. ENTOMOLOGY

Thursday, May 13—2 to 4:30 p m  
Departmental Auditorium, Main Hall

### THE ANOPHELINE VECTORS OF MALARIA OF THE WORLD

WILLIAM H. W. KOMP, *Division of Tropical Diseases, National Institute of Health, United States Public Health Service, Bethesda, Md.*

At the present time, more than 200 species and subspecies of anophelines are known throughout the world. More than 50 of these are important vectors of malaria. In a brief paper, it is obviously impossible to consider each species separately. Reference is therefore

criteria of vector ability, and the methods of determining it are discussed. The important vector species, their distribution, and refer

195 species and subspecies, as known in 1926, is given. Four years later a second paper (Covell, 1931) reviewed the work done in the

the errors in these, and in other later articles. Weyer lists 46 dangerous vectors and 24 relatively unimportant carriers.

Since 1940, stimulated by the need for information caused by World War II, a number of papers have appeared dealing with anopheline taxonomy, vector abilities, and distribution. Simmons and Aitken (1942), in *The Anopheline Mosquitoes of the Northern Half of the Western Hemisphere and of the Philippine Islands*, give data on natural and artificial infections in the species of these areas. Farner

and Mackerras (1947) cover the vectors of the Australasian region

It is very necessary to draw attention to the high incidence of malaria around Graz (p. 103) occur, if they exist

(not summers and presence of carriers) severe—outbreaks may

# SUMMARY

A number of small active foci of tertian malaria in Austria are described. Due to the fact that *A. maculipennis* and other species of anophelines are common in Austria, it is pointed out that larger outbreaks of tertian malaria may develop if suitable conditions occur.

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separable on egg characters; otherwise they are practically indistinguishable. These kinds differ in choice of breeding place, avidity for human blood, sexual behavior, and in malaria transmission. They are further separated by *sterility barriers*, sometimes complete, sometimes sho

question

Bates (11 status. The matter is also discussed by Buxton (1938), who mentions other examples. He says, "Our comprehension of a 'species' is undergoing continual extension and becoming more and more difficult to define. We might well be advised to record the facts and refrain from making further categories or definitions." Evans (1938) also discusses the subject as applied to mosquitoes and arrives at pessimistic conclusions with regard to its impact on classical taxonomy. Species complexes recently worked out are the *maculipennis* complex of the western part of the United States (Aitken 1945), the *tarsimaculatus* complex of Central and South America (Rozeboom and Gabaldon

But for the is one

on any of the usually accepted morphological characters, but which differs in its ecology, food preferences, and, in *Anopheles*, in its ability to transmit malaria. Some examples of such races are the

Other similar instances have been noted in India by Senior (1947). The taxonomist can offer no assistance in such cases, as the factors entering into the formation of such races belong to the field of genetics and physiology.

*Determining vector ability*—The most usual method of incriminating a species as a vector is dissection to ascertain whether it is infected with malaria. The published data, even for the same species, are often contradictory. Several factors may influence the results. Often it is not stated whether stomachs only, or salivary glands only, or both, were found in a dry or humid or warm should be noted,

particularly in epidemics, The place of capture of the as those taken in houses are

Corell (1944) again brought up to date the information on the Keys to the An of the Indian area and of the Far East. Mosquitoes of the World, by Russell, Rozeboom, and Stone states the relation to malaria of the known vectors through world Chapter 8 of Practical Malariology, by Russell, We Manwell (1946), is one of the best recent treatments of the s as the distribution of vectors is given in compact form by cont and political subdivisions of the world, with a list of 54 chief re *General considerations*—Before considering the various sp and the areas in which they are vectors, certain general aspects of whole problem of malaria vectors should be discussed *Taxonomy*—The first of these items is taxonomy, which deals w the names applied to the mosquitoes which transmit malaria. Nan are convenient labels for designating the mosquitoes concerned malaria transmission. The function of the taxonomist is to suppl the proper labels. The subject is in a state of grave confusion Much of the early taxonomic work must be discarded, owing to the imperfect state of knowledge at the time. Many new species and sub species have been described, and many old species have been found to consist of "complexes." It is often necessary to reinterpret the older names in the light of the newer knowledge *Relative to the problem of malaria vectors, many malarialogists, who were not primarily taxonomists, misidentified the species they were investigating. Much of the early data on vectors is worthless because the species involved is not known with certainty. Often different names are applied to the same species. The Dutch in the East Indies use a nomenclature which differs from that of English speaking workers. An additional complication is that there are no generally accepted definitions of 'species' and 'sub species'. Their meanings are different for the museum taxonomist and for the field worker. The terms "species," "sub-species," "varieties," and "races" are used, with no uniformity in their application. As an aid to future research, it is suggested that investigators of the vector ability of anophelines take advantage of the knowledge of all stages of the life history, including the eggs, should be forwarded, with precise data as to locality, etc. The material could then be conserved for future study. The work of the taxonomist is indispensable to the study of any disease carried by an intermediate host, as without correct names as labels, discussion of the subject would result inextricable confusion. *Species-complexes and biological races*—The discovery of species complexes in units which were earlier considered to be a single species complicated the already confused subject of anopheline taxonomy. The classic example is the *maculipennis* complex of Europe, which is too well known to be discussed extensively. Suffice it to say that six or more kinds of *maculipennis* are*

of their great numbers, which offset their low infectivity and their usual zoophilism (Russell and Rao 1912). Second, they may become infected during an epidemic begun by a more potent vector, and may perpetuate such an epidemic. In Sumatra, Walsh and Walsh Sorg drager (1921) found that *A. hyrcanus sinensis*, not regarded as an efficient vector there, became infected during the course of an epidemic to such an extent that the actual percentage of infective *hyrcanus* was greater than that of the primary vectors (*lochii* and *sundaicus*), although the natural infection rate in these was much higher. A similar instance involving *A. amictus hillii* in Australia is quoted by Covell (1914).

*Important vectors of malaria, their distribution, and references to the literature*—In the following table, the writer acknowledges his debt to the publications of Russell, Rozeboom, and Stone (1913), and of Russell, West, and Manwell (1916). He followed their method of tabulation to enable him to present the data in a concise form. The indispensable "Review of Applied Entomology, Series B," was consulted in obtaining pertinent references.

TABLE 1—The Anopheline vectors of the world arranged by geographical areas

| Species   | Distribution   | Authorities  |
|---|--|--|
| UNITED STATES   |  |  |
| <i>quadrimaculatus</i>  | Texas to New Hampshire. Northern states west to Minnesota.   | Barber, M. A., Kemp, W. H. W., and Hayne, T. B. 1927. King, V. 1929.   |
| <i>maculipennis freeborni</i>   | Arid southwest. California.  | Barber, M. A. and Forbush, L. R. 1933.                                 |
| Minor or suspected vectors <i>crucians punctipennis albimanus walkeri</i> |  |  |
| MEXICO  |  |  |
| <i>albimanus</i>  | Gulf coast and southern tropical lowlands.   | Hoffmann, C. O. 1935.  |
| <i>pseudopunctipennis</i>   | Interior plateaus.   | Verrill, L., Casts, S. O., and E. W. C. 1941.                          |
| <i>darlingi</i>   | States of Chiapas and Campeche.  | Laessle, J. 1947.  |
| CENTRAL AMERICA   |  |  |
| <i>albimanus</i>  | Tropical lowlands throughout S. British Honduras. Gulf of Honduras region, E. Guatemala. Highlands of Guatemala. | Simmons, J. R. et al. 1939. H. W. and Ruiz, H. 1929.                   |
| <i>darlingi</i>   |  | Kumm, H. W., and Rem, L. M.  |
| <i>pseudopunctipennis</i>   |  | Olaquinto Mira, M. 1936.   |
| Minor or suspected vectors <i>punctimacula vestitipennis</i>              |  |  |
| WEST INDIES   |  |  |
| <i>albimanus</i>  | Greater Antilles. Jamaica.   | Carr, H. P., and Hill, R. Boyd, M. F., and Arls, F. Earle, W. C. 1930. |
| Minor or suspected vectors <i>crucians vestitipennis grabbamii</i>        |  |  |

Experimental infections are not now used so much as formerly, and less emphasis is placed on the results of such infections. They may show variations in the susceptibility of different species, and should always be made with a good vector as a control, to eliminate the effect of possible variations in infectivity of the gametocytes. Experimental infections cannot show the natural capabilities of a species, as other factors may be of more significance.

*Criteria of vector ability*—Various criteria of vector ability have been used. Rice and Barber (1937) critically examined the factors involved. Susceptibility to malaria infection, attraction to man, occurrence in houses, and a relatively high rate of salivary gland infection have been held to be evidence of vector ability. But these authors stated that no single one of these criteria is sufficient to incriminate a species. On the basis of their studies in Greece, they

malaria rate in Egypt, *A. pharoensis* is highly attracted to man, but has a low sporozoite index, and is not found frequently in dwellings. It is able to maintain only a low malaria endemicity. Some species are easy to incriminate, as was *A. gambiae* in its invasion of northeastern Brazil for it satisfied all the criteria. In certain other circumstances, incrimination is easy, as in the plateau region of central Mexico, where *A. pseudopunctipennis* is the only species present in an area of endemic malaria. Many other cases are difficult to establish and require long study over a period of years. The parasite index of infants may be of use, if the abundance of several possible carriers varies from year to year, or in locality, or in season of year. The infant parasite rate can be correlated with the abundance of the suspected vector. Spleen and blood surveys, even when made throughout the year, do not always indicate the vector species. Malaria may be due to a species not common at the time of the survey, while another species may be common then, but is not the carrier. The true vector may have been abundant some months or years before, and

*Primary and secondary vectors*—Anophelines as vectors fall into two classes. The first contains those species universally known to be dangerous wherever found. There are probably not more than 15

in this category. The second class consists of species which are ordinarily innocuous but which under certain conditions may become vectors. These secondary vectors may be important, first, because









# V MALARIA

TABLE I—The Anopheline vectors of the world etc.—Continued

| Species  | Distribution  | Authorities  |
|--|---|--|
| BURMA MALAYA INDO-CHINA SIAM SOUTH CHINA AND FORMOSA   |   |  |
| <i>acronitua</i>   | India Ceylon Burma S W China Siam Indo-China Malaya Neth-lands Indies Borneo Celebes                                    | Genetray J Toumanoff C and Try H T 1937  |
| <i>culicifacies</i>  | India Ceylon Burma S W China Siam Tonkin Arabia S W China   | Gaschen H 1934   |
| <i>hyrcanus sinensis</i>   | Assam Burma, Indo-China Neth-lands Indies N and S China Japan, Korea Formosa  | Robertson R C 1940 Gaschen, H 1936   |
| <i>leyporensis candidiensis</i>  | India, Burma S China Indo-China Formosa   | Jackson R B 1934 Robertson R B 1941 Yao Y T 1943   |
| <i>leucophrys</i>  | India Ceylon Burma, Siam Indo-lands Indies, Borneo  | Clark R H P., and Choudhury M A 1941 Stoker W J 1934   |
| <i>maculatus</i>   | India Ceylon Burma, S China Siam Indo-China Netherlands Indies Formosa Philippine Is-                                   | Robertson R C 1940   |
| <i>minimus</i>   | N and E India Ceylon Burma Assam Siam Indo-China S China, Formosa   | Raynal J and Gaschen H 1935, Chang T L 1941 Fenz L O 1937 Sweet, W C Fenz L O Chow C Y, and Hsu S C 1942   |
| <i>sundicus</i>  | India Burma, Siam Malaya Neth-lands Indies  | Jackson R B 1936   |
| <i>umbrosus</i>  | E India Tonkin Corbin China Malaya Netherlands Indies Borneo Celebes  | Walch E W and Walch-Sordrager B 1941, Stokins V A 1927 Far-ber M A 1918, Walch E W and Somlo R 1940 Overbeck J O and Stoker W J 1937   |
| Minor or suspected vectors <i>annularis</i> , <i>baczi</i> , <i>leyporensis</i> , <i>leyporensis</i> , <i>larwari</i> , <i>borumbrosus</i> , <i>separatus</i> , <i>splendens</i> . |   |  |
| NETHERLANDS EAST INDIES  |   |  |
| <i>acronitua</i>   | See section on Burma, etc   | Overbeck J O and Stoker W J 1937, Genetray J Toumanoff C and Try H T 1937 Small, F H 1937 Farner D S 1943, Hestlin, L F and Johnston, R S 1943 Ewellmeyer, R H and Rodenwaldt, L 1937 Farner D S et al. 1946 |
| <i>albivittatus</i>  | India, Ceylon, Burma, Siam Indo-China, S China, Malaya, Neth-lands Indies, New Guinea, Phil-ippine Islands              | Gaschen H 1934, Hodgkin, E F 1937 Fomlo R 1935, Farner D S 1943  |
| <i>reanus nigritimus</i>   | India, Ceylon, Burma, Siam S China, Indo-China, Malaya, Netherlands Indies, Borneo I bil Sumatra, Java, Celebes, Borneo | Venhuis, W O 1940  |
| <i>reanus X</i>  | India, Ceylon, Burma, Siam S China, Indo-China, Malaya, Netherlands Indies, Borneo I bil Sumatra, Java, Celebes, Borneo | Walch, E W and Walch-Sordrager B 1941 Doorenbos, W J 1934  |
| <i>reanus</i>  | India, Ceylon, Burma, Siam S China, Indo-China, Malaya, Netherlands Indies, Borneo, Phil-ippine Islands                 | Doorenbos, W B 1931 Stoker W J 1934  |
| <i>reanus</i>  | See section on India, etc   | Doorenbos, W B 1931 Venhuis, W O 1941 Farner D S 1943, R 1943  |
| <i>reanus</i>  | do  | Overbeck J O and Stoker W J 1937 Venhuis, W O 1942   |
| <i>reanus</i>  | Java, Bali, Borneo, Philippine Is-lands   | Walch E W and Walch-Sordrager B 1921 Overbeck J O, J., and Stoker W J 1934   |
| <i>reanus</i>  | See section on Burma, etc   | Walch E W., and Somlo R 1940 Overbeck J O and Stoker W J 1937  |
| Suspected vectors <i>subpictus</i> , <i>lowlandus</i> .  |   |  |

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## ACTIVE AND PASSIVE DISPERSION OF ANOPHELINE SPECIES

A. L. AYROZA GALVÃO,<sup>1</sup> *Adjunct professor of the Department of Parasitology of the Faculty of Hygiene and Public Health of the University of Sao Paulo, Brazil*

By dispersion of an anopheline species is understood the phenomenon of its dissemination in a given area, which can be due to seasonal meteorological factors, or to its invasion of a new territory resulting from artificial conditions created therein, or to transportation facilities evolved from the rapid modern means of locomotion. This being the case, dispersion should be considered active or passive and can be studied under the following headings:

(1) Active dispersion

Dispersion by flight

Dispersion by propagation from breeding place to breeding place

(2) Passive dispersion

Dispersion of anophelines in the aquatic stages.

Dispersion of adult anophelines

Active dispersion by flight

As early as the beginning of this century dispersion by flight attracted the attention of several authors, such as James (1903), Ross (1905), Stephens and Christophers (1906) and others, who usually calculated flight capacity by measuring the distance between sites of adult captures and the nearest breeding places or by determining the maximum distance between breeding places and localities in which cases of malaria were found. Such a procedure is called the "range". Another procedure used for the first time by (1916) in Panama. They released *A. albimanus* and *A. tarsimaculatus* (= *A. aquasalis*), which

attempt to find resting places where their ovaries can mature. James believes that this flight takes place before the first blood meal. Such flights sometimes occasion large mass removals and have an impor

<sup>1</sup> The author is indebted to Prof. J. Lane and Dr. G. Hayes for the translation of this article into English.

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At this point in the meeting Prof V H Swellengrebel presented the report of the committee appointed on May 10 to consider the possibility of the permanent fusion of the Congress on Tropical Medicine with the Congress on Malaria. The report was unanimously approved by the Section and referred to the Committee on Resolutions of the Congresses. (See Resolution I, as adopted in the closing plenary session.)

protected in areas reached by the anopheline vectors during years more favorable for propagation. In relation to *A. darlingi* we observed in Araraquara, São Paulo, Brazil, that the DDT-residual spraying of houses does not diminish the production of larvae in the nearby breeding places. The same has been noted by Dr. A. Vargas (personal communication) in regard to *A. darlingi* in Ribeirão das Lages, in Rio de Janeiro.

The literature on anopheline dispersion is vast. Eyles (1944) made an extensive review of this subject based on practically all important papers up to that time. We condensed his data in table 1 and added some of our own, mainly from publications subsequent to his monograph. A summary of the more important items from the latter are

... based on the observation method ... *lingi* were ... were captured. In certain cases, however, he noted that the nearest breeding places were located at 1.0, 1.5, and 2.0 kilometers away (0.65, 0.94, and 1.25 miles).

Coutinho (1942) captured at Jacarepaguá, State of Rio de Janeiro, ... and abundant numbers ... breeding ... between. Recently Dr. Correa and his collaborators (Correa et al., 1948) in experiments made with mosquitoes stained with methylene blue, and ... State of

... had been caught in houses and stained with bronze powder, as ... captured 21 of those stained specimens on the island in the next few days

Vargas (1928) controlled malaria transmitted by *A. tarsimaculatus* (= *A. aquasalis*) in a camp of more than 5,000 persons working at a hydroelectric plant at Cubatão, São Paulo, by clearing a 500 meter belt of vegetation and breeding places. Gillette (1946), by placing down traps spaced at intervals of half a mile from the breeding places,

tant bearing on the dispersion of species. The third type of flight is that directly related to malaria transmission and is of great importance in the dispersion of species and in the delimitation of malaria control areas. For this purpose we should distinguish the maximum flight from the maximum efficient flight range of an anopheline. The latter corresponds to the distance from breeding places at which anophelines are found in sufficient number to cause malaria transmission.

To such types of flight we should add the seasonal dispersion related to hibernation in temperate regions, and seasonal flights of anophelines found in tropical regions which are correlated with the dry and rainy seasons. Both flights can attain long distances. In Table I distances of hibernating flights are noted such as that of *A. sacharovi*, 12 kilometers (7.1 miles), stated by Kligler (1924) and born (1929) and of *A. maculipennis freeborni*, 6 kilometers (4.1 miles), by Gattrell et al (1916). The withdrawal of anophelines to drought-resistant breeding places in hot countries during the dry season and their consequent dispersion at the beginning of the rainy season are an epidemiological equivalent to flight related with hibernation, though biologically different. Curry (1934) notes a 12-kilometer (12 mile) flight of *A. albimanus* in Panama at the start of the rainy season and De Verteuil (1931) mentions a migration of *A. farautensis* (=*A. aqualis*) of 4 kilometers (2.5 miles) in Trinidad. The anopheline invasion of new areas due to changing conditions brought about by engineering or agricultural projects is identical with seasonal dispersion.

In practice the different types of flight are sometimes difficult to distinguish but the measurement of oviducts can be used to demonstrate whether the anopheline is nulliparous or hibernating. Anopheline flight depends on several factors, such as the species, the production of breeding places, relative humidity and temperature, direction and velocity of prevailing winds, topography and distribution of intermediate blood supply and resting places. Without going into details, we wish to call attention to the fact that some authors have noted that certain anophelines, at least, fly against the wind (Le Prince, 1912, Le Prince and Oren, 1916, Shapiro et al, 1944, and recently Correa et al, 1948) while others have noted the contrary (Swellengrebel, 1929, 1934, and Santiago 1931, and others). The importance of seasonal dispersion of anophelines in hot countries and breeding places are left untreated. Dispersion is limited only by the annual variation of meteorological conditions. In such cases it is difficult to estimate the necessary extent of DDT spraying of the houses so that populations can be



In World War II dispersion by propagation was frequently observed when new conditions were created, such as emergency roads, deforestation, bomb craters, and abandoned foxholes. These provided many breeding places for anophelines as Perry (1946) and Oman and Christensen (1947) noted in the South Pacific. Dispersion by propagation was also observed in the spreading of *A. gambiae* over the northeast of Brazil as described by Shannon (1932), Barber (1940), and Soper and Wilson (1943). Cora Garcia (1943) described the dispersion which *A. darlingi* and *A. albimanus* have made into the Venezuelan hinterland spreading on recent geological formations and avoiding the eocene and older formation. Lewis (1944) believes that the *A. gambiae* introduction into Wadi Halfa, in Anglo Egyptian Sudan, was carried out by propagation and not by land vehicles or boats.

Komp (1940) described the occurrence of *A. darlingi* in British Honduras and Guatemala. The fact that this species was never found in other Central American countries north of Panama leads to the hypothesis of a long distance migration of this anopheline. Further research must be carried out in order to clarify this problem.

#### PASSIVE DISPERSION OF ANOPHELINES IN THE AQUATIC STAGES

The passive dispersion of anophelines in the aquatic stages is effected by natural means such as floods and cloud bursts. Dr. O. Silva

Vargas (1948) found larvae of *A. darlingi* in a small flooded margin which is also brought we frequently  
Larvae can

#### PASSIVE DISPERSION OF ADULT ANOPHELINE

This mode of dispersion is effected by land, aquatic and air transport. In an era in which the means of locomotion have become increasingly rapid and cover a wider radius, the importance of passive dispersion from one focal region to another is great.

mosquito transportation in trains and other vehicles. Thibault (1910) noted that *A. quadrimaculatus* traveled 40 miles in a carriage. Eyles (1945) mentions this species as traveling 100 miles in an automobile.

observed that this species, in Trinidad, could fly 48 kilometers (30 miles). *A. osvaldoi* flew only half a mile from its breeding places (800 meters).

The species of *Kerteszia* seem to have a short flight range. Dr. Correa (personal communication) found the breeding places of *A. cruzi* in bromeliads 50 meters distant from the houses in which infected specimens were previously captured by him at Serra do Mar.

As to the other species, recent publications confirm the data of previous authors. Shapiro et al (1944) and Russell et al (1944) studied the effect of wind on dispersion of anophelines, Drosdova (1941), Smetanina (1942), Daggy (1945), and Eyles and Bishop (1946) studied dispersion over channels, rivers, or the sea, Daggy (1945), Drosdova (1941), Ivanova (1942), and Shapiro et al (1944) studied flight range by several methods.

The data are summarized in table 1, which gives flight range of anophelines of the world. Most of these data were taken from Eyles (1944). Since a large number of publications cited by him were not accessible to the writer, the origin of such references is given in the bibliography.

#### ACTIVE DISPERSION BY PROPAGATION

Propagation from breeding place to breeding place is the principal mode of dispersion of anopheline species "in natura," and is directly influenced by all of the factors involved in the flight capacity of anophelines. Its importance as a mode of dispersion is relatively

limits of an area to be controlled

high  
can

usually has a  
frequently  
by Grieco

(1943) and also observed by the author, which occurred in the neighborhood of the city of São Paulo, in 1941, where malaria had never been observed. The same

abundant. The same phenomenon was observed in 1941, when it was

able to reproduce normally. Insect transportation by aircraft has also been studied by Soper and Drees (1935), MacLeod (1939), and Whitfield (1933), and Whitfield (1933), and Whitfield (1933).

planes arriving at Recife, Natal, Fortaleza, and Belém airfields were 0.17 per plane in 1943, dropped to 0.001 in 1944, and to 0.0006 in 1945 during which period the total numbers of planes inspected were 1,552, 2,628, and 4,930, respectively. With the constant improvement in insecticides, even better results may be expected. Despite this fact, aviation continues to be a potential mode of insect transportation from one region to another throughout the world. Soper and Wilson (1943) call attention to certain difficulties in plane mosquito control. Miller et al. (1947) state that only 10 percent of the insects present in planes are found by the inspectors. In considering the number of mosquitoes found in all planes at North American airports and correcting for this 90-percent deficiency, they considered it a possibility that 10 to 25 anophelines might have landed in the United States. They believe that such a number is not dangerous, due to local climatic conditions and airport sanitation measures. We feel that such facts demonstrate

regions of tropical

during war time. For this reason we believe that maximum importance should be attached to the international agreements concerning aircraft insect control, wherein obligations and responsibilities are clearly defined.

Table 2 contains a list of anophelines found in planes arriving at airports of several countries, and is compiled from data available to the writer. Whitfield's bibliography was extensively used as source material.

TABLE 1—Some data on flight range of the anophelines of the world

| Anopheline spp.        | Maximum observed flight range <sup>1</sup>                     | Maximum flight proved by experiment <sup>2</sup>                          | Maximum noted seasonal flight <sup>3</sup> |
|------------------------|--|---|--|
| <i>A. acutus</i> —     | 530 (0.33) Bore (1934)   | 430 (0.2) Mangkoewinoto (1923)<br>900 (0.56) Ave Lallemon et al. (1931)   |  |
| <i>A. albimanus</i>    | 1,600 (1.0) Le Prince (1912)<br>800 (0.5) Howard et al. (1912) | 1,600 (1.0) Zetek (1915) —<br>1,880 (1.17) Le Prince and Orenslein (1916) | 19,200 (12.0) Curry (1934)                 |
| <i>A. albivittatus</i> | 3,000 (1.67) Coutinho (1942)                                   | 1,800 (0.94) Correa et al. (1943)   |  |
| <i>A. algeriensis</i>  | 400 (0.23) Telles (1939)<br>1,500 (0.94) Enkolopov (1944)      | —   |  |

See footnotes at end of table

In Brazil, Coutinho and Ferraz (1946) captured 593 anophelines specimens (among them three *A. darlingi*, one of which was infected) inside night trains in Minas Gerais, during a 4 months period of observation. Soper and Wilson (1943) refer to *A. gambiae* and *Aysorhynchus* species found in vehicles inspected at the border of the gambiae invasion area. Deane (1947) found *A. darlingi* in trains, autos, canoes, and ships in Amazonia.

Most authors consider that *A. gambiae* was introduced into Brazil by rapid postal steamers (aviso) which took less than 4 days to travel from Dakar to Natal. In favor of this opinion there is the strong argument that when Shannon first found *A. gambiae* its foci were about 500 meters from the place where the aviso docked, whilst the airfield was a few kilometers distant, making it improbable that gambiae came in the few airplanes which had crossed the Atlantic prior to that time.

*A. gambiae* was introduced into Brazil from West Africa, causing a malarial epidemic which (annual report for 1945) was quite probably greatly increased during the war. Lewis (1942) stated that the northern limit of this species

development of *A. gambiae*, and the human population is at the most sparse (personal communication to the writer by Dr P. L. Soper). This species was eradicated in Egypt by cooperation between

of insects, vectors of disease and pests by aircraft. Since that time many papers have been published on this subject. Griffiths and Griffiths (1931) showed that live *Aedes aegypti* could be transported great distances in airplanes. Sicel et al (1939) made identical observations of *A. gambiae* transported by airplane from French Sudan to Marseille. These mosquitoes arrived in good condition and were

TABLE 1—Some data on flight range of the anophelines of the world—Continued

| Anopheline spp                   | Maximum observed flight range *   | Maximum flight proved by experiment †  | Maximums noted seasonal flight ‡  |
|----------------------------------|---|--|---|
| <i>A. maculatus</i> ...          | More than 800 (0.5) Strahan (1940)  |  |   |
| <i>A. maculipennis</i>           | 2 000 (1.25) Sargent and Ferrent (1905)<br>2 400 (1.5) Morris (1915)<br>2 000 (1.25) Robertson (1930) | 2 500 (1.56) Sella (1920)<br>3 500 (2.19) Missiroli (1927)<br>7 000 (4.34) Ottolenghi et al (1929)   | 18 000 (11.25) Shipov (1926)<br>4,800 (3.0) Markovitch (1942)   |
| <i>A. parvus</i>                 |   | (1922)<br>14 000 (8.7) Swellengrebel and Nykamp (1934)<br>8 500 (5.44) Hill et al (1935)   | 8 400 (4.0) Freeborn (1932)   |
| <i>A. maculipennis freeborni</i> |   | 900 (0.56) Russell and Santiago (1934 a)   | 1 600 (1.0) Ramsay (1930)<br>12,800 (8.0) Manson and Ramsay (1934)  |
| <i>A. menggenus</i>              |   |  |   |
| <i>A. minimus</i>                | 1 600 (1.0) Harrison and Hensley (1933)<br>800 (0.5) Rice (1935)                                      |  |   |
| <i>A. minimus ferrenti</i>       | 2,400 (1.5) Manalang (1931) *   | 2 200 (1.4) Russell and Santiago (1934 b)  |   |
| <i>A. multicolor</i>             | 4 000 (2.5) Craig (1900) *  |  |   |
|                                  | 2 100 (1.3) Kilgler (1924)  |  |   |
|                                  | 12,800 (8.0) Kirkpatrick (1925)   |  |   |
| <i>A. neomaculipennis</i>        | Short distance De Ver-teuil (1931)  |  |   |
| <i>A. pulcherrimus</i> ...       | 25,000 (15.5) Wright (1915)<br>3,200 (2.0) Christophers and Shortt (1921)                             |  |   |
| <i>A. rondoni</i>                | 800 (0.5) Davis and Shannon (1928)  |  |   |
| <i>A. sacharovi</i>              | 2 400 (1.5) Barraud (1921)<br>4 800 (2.8) Kilgler (1924)  | 2 700 (1.7) Guger et al (1919)<br>1 270 (0.79) Barber and Hayne (1924)<br>645 (0.4) Kuman (1929)<br>1 000 (0.65) Carpenter (1930)<br>220 (0.14) Weatherbee and Hasell (1939)<br>670 (0.41) Smith et al (1941)<br>4 600 (2.8) Eyles and Bishop (1943) | 8 000 (5.0) Kilgler (1929)<br>8 800 (5.5) Reiter and Sahiternik (1929)<br>14 000 (8.7) Kilgler and Mer (1930)<br>12 000 (7.5) Kilgler (1937)<br>5 000 (3.68) Kilgler (1928) |
| <i>A. sergenti</i>               | 2 500 (1.56) Kilgler (1924)   | 4 000 (2.5) Shapiro et al (1944)   |   |

all and

See footnotes at end of table





TABLE 1—Some data on flight range of the anophelines of the world—Continued

| Anopheline spp  | Maximum observed flight range  | Maximum flight proved by experiment   | Maximum noted seasonal flight   |
|---|--|---|---|
| <i>A. stephensi</i>   | 800 (0.5) Stephens and Christophers (1927)<br>800 (0.5) Mulligan and Bailey (1935)<br>2,400 (1.4) Ashfi and Ma (1938)<br>400 (0.25) Telles (1939)<br>800 (0.5) James (1942)  | —   | —   |
| <i>A. sticticus</i><br><i>A. subpictus</i>  | 1,200 (0.75) Christophers (1944)<br>1,600 (1.0) Swellengrebel and Swellengrebel da Graaf (1919)  | 1,500 (0.94) Aze Lallemonet et al (1932)<br>720 (0.45) Ratanarayana (1934)            | —   |
| <i>A. subpictus</i> Indef<br><i>A. sundicus</i>   | 800 (0.5) Christophers (1913)<br>800 (0.31) Schnodder et al (1919)<br>1,600 (1.0) Swellengrebel and Swellengrebel da Graaf (1919)<br>2,000 (1.27) Van Breeman (1919)<br>2,000 (1.25) Tee Porten (1944)<br>2,000 (1.25) Correll (1944)<br>4,800 (2.8) Schurman et al (1944)<br>2,400 (1.5) Syengar (1931)<br>2,400 (1.5) Bernard (1944)<br>7,000 (4.3) Niles (1944)<br>2,400 (1.5) Kilgus (1944)<br>2,400 (1.5) de Souza (1944)<br>2,400 (2.0) Curry (1944) | 2,000 (1.25) Russell and Santiago (1934 b)<br>6,200 (4.0) Van Breeman (1920)          | —   |
| <i>A. superpictus</i>   | 1,600 (1.0) Earle (1932)<br>1,200 (0.75) Fisher (1934)<br>4,800 (3.0) Gilett (1940)  | 1,200 (1.1) Zetek (1915) and Le Prince and Ornstein (1915)                            | 4,000 (2.5) De Verteuil (1931)<br>4,800 (3.0) De Verteuil and Spence (1937) |
| <i>A. tarsatorius</i>   | 1,200 (0.75) Mulligan and Bailey (1935)<br>(Considerable distance Barber (1914))   | 1,000 (0.62) Aze Lallemonet et al (1932)<br>1,400 (1.2) Russell and Santiago (1934 a) | —   |
| <i>A. tritaeniorhynchus</i><br><i>A. tritaeniorhynchus</i><br><i>A. tritaeniorhynchus</i> | 2,200 (2.0) Bang et al (1943)<br>2,200 (2.0) Bang et al (1943)<br>Lewthwaite (0.71) Strickland (1924)<br>Lewthwaite (0.20) Strickland (1924)   | 800 (0.5) Aze Lallemonet et al (1932)   | —   |

Number in parentheses, second in miles, of the maximum flight range recorded by each author.  
 Subspecies *A. crucians* Bradley.  
 Minimum.





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# ADAPTABILITY OF EXOTIC MALARIA PARASITES TO INDIGENOUS ANOPHELINES

MARTIN D. YOUNG, *Division of Tropical Diseases, National Institute  
of Health, United States Public Health Service, Columbia, S. C.*

## INTRODUCTION

The knowledge of the definitive host parasite relationships between the various strains of human plasmodia and the different species of anopheline mosquitoes is meager and scattered. In many malarious areas of the world, the insect vectors for the indigenous strains of malaria have been determined, often on epidemiological grounds. But even in such areas, there still remains much to be determined about the efficiency of the vector parasite associations.

At the beginning of World War II, it became obvious that malaria plasmodia indigenous to various areas of the world would be disseminated throughout other areas due to the large scale and rapid movement of troops who were infected with malaria. Upon being introduced into a new area with its characteristic and often different anopheline fauna, the exotic plasmodia would then be exposed to a insect vector which might be different from its former vector. The question of the compatibility of the new strains of plasmodia to the indigenous vectors then became an important one. Would the relationship be more efficient than previous combinations, less efficient or about the same?

It was known only that some new combinations of parasites and vectors might be either more or less efficient than the old combinations. But there was not enough information available on such new parasite vector relationships to obtain an accurate forecast of the problem in this country. Therefore, investigations along this line were carried on during the war. The results of this work and the compilation of similar work by others will be reported in this paper.

## OBSERVATIONS ON "PLASMODIUM VIVAX"

To determine whether one strain of malaria is more infective than another strain to one species of mosquito is rather difficult. Infectivity of a patient at any one time apparently depends on diverse factors such as the stage of the disease and the maturity of the gametocytes present. An individual patient might infect mosquitoes on one day and not do so on another. Thus, limited data are not trustworthy, particularly if the results are negative.

If a fairly large amount of infectivity data is available, it is possible to evaluate the infectiveness of various strains by comparing the percentages of mosquitoes infected, the intensity of oocysts

portance is greater now probably than ever. I am glad to say the French authorities are truly aware of the importance of increased traffic by air and truck across the Sahara.

Dr SAADALAH MADWARI (Egypt). I should like to emphasize some points about the passive transportation of mosquitoes in eradication campaigns. It seems to me that attention is mainly concentrated on aerial transport of mosquitoes. One can readily understand that; however, one should not ignore water transport of insects by boats, which is also very important and more difficult to control. In the invasion of Gambiae to Brazil or Egypt, it is almost certain that water transport by boats was the means taken for the invasion.

There is another very important point which was raised by the paper, and that is the passive transportation of mosquitoes by wind.

There is positive evidence from the Gambiae eradication campaign in Egypt, that mosquitoes could be transported by wind for a distance of over 70 kilometers. Thus in undertaking eradication campaigns it is worthy of consideration to study the wind transporta-

some elaborate piece of work.

oocysts and sporozoites. Most oocysts developed to maturity. Also the incubation period in the mosquito was relatively short, averaging about 11 days or less at 75° F. Transmission to patients was readily effected by infected mosquitoes, indicating viability of the sporozoites. This evidence also indicates that *A. quadrimaculatus* was a very favorable host to the various strains of malaria.

One test of the adaptability of exotic parasites to indigenous mosquitoes is the ability to maintain these strains by continuous mosquito passages. As pointed out above, the indigenous mosquito is a new vector host for the foreign parasites, and it was necessary to determine whether the exotic parasites would retain their virulence upon repeated passages through this new vector.

One vivax strain from the China Burma India theater, one from the Mediterranean area, and about 12 from the Pacific area were maintained for several, and in some instances many, mosquito passages. The species used was principally *A. quadrimaculatus* of the Pacific vivax strains (Chesson or V-1027-NG) has been maintained for almost 4 years, involving many mosquito and blood passages in hundreds of patients.

There was no evidence of any lessened virulence in any of the strains after continued passage. Apparently, these exotic malarias adapted themselves readily to *A. quadrimaculatus* as a host vector. The considerable data also on passages through *A. m. freeborni* with indication that it also is a good host-vector and probably better than *A. quadrimaculatus*.

However, all species of anophelines do not show a similar susceptibility to vivax malaria. It is relatively easy to determine the relative susceptibility of various species of mosquitoes to a particular strain of malaria. This can be done by feeding the various mosquitoes simultaneously on a malarious patient and incubating the potential infected mosquitoes under similar conditions. Using this method, employing *A. quadrimaculatus* as a control, marked differences in the susceptibility of various American anophelines to the vivax malarias were demonstrated. The data from this and other laboratories on such comparative feedings are shown in table I.

Compared to *A. quadrimaculatus*, Young et al. (1946) found that *A. punctipennis* had about the same susceptibility to certain vivax malarias, *A. pseudopunctipennis* and *A. maculipennis* about one-fifth as susceptible, and *A. albimanus* about one-fiftieth as susceptible. The important vector of malaria on the west coast of the United States, was significantly more susceptible to foreign vivax malarias than was *A. quadrimaculatus* (Young and Burgess 1946).

Experimental work now underway indicates that the relative susceptibility of the various mosquitoes to a domestic strain of vivax probably will be similar to that found with foreign malarias.

Boyd et al. (1933) also found that vivax malaria from

zoites in the infected specimens, and the maintenance of virulence through continued man mosquito passages.

*P. vivax* malarias from the tropical war zones were brought into the United States by returning troops. We tested the infectivity of these exotic malarias to American anophelines. *Anopheles quadrimaculatus* was chosen as the standard testing species.

It was found that *vivax* malaria originating in the areas of the Southwest Pacific, Mediterranean, Liberia, China Burma India theater, and the Caribbean all infected *A. quadrimaculatus* (Young et al., 1946, 1948). Some of the feedings on malarias from Guadalcanal, New Guinea and the Mediterranean areas showed 100 percent of the mosquitoes infected. One lot of mosquitoes fed upon a *vivax* malaria patient from the China Burma India area showed 94 percent infected.

percent of 1,306 mosquitoes and 129 Pacific cases infected 20.9 percent of 4,920 mosquitoes (Young et al., 1948).

Malarias from the Caribbean and Liberian areas did not give as high a maximum infectivity or as high an over all infection rate, but as only seven cases were exposed to mosquitoes, this comparison may not be valid. It is quite likely that mosquitoes were not fed at an optimum time.

The above findings relate to mosquitoes infected by clinical relapsing patients. *A. quadrimaculatus* were fed also on patients showing asymptomatic parasitemias. In these patients the parasites were present but not in quantities sufficient to produce symptoms. Also, the gametocytes were fewer than in the clinical relapsing patients who had higher total parasite counts.

Of 20.9 mosquitoes fed upon the asymptomatic patients, 11.6 percent were infected (Fyles et al., 1948). The average number of oocysts per infected gut was 148. The results with malarias from the Pacific and the Mediterranean areas were similar.

That the low grade parasitemias infected mosquitoes is another indication that *A. quadrimaculatus* was a very favorable host to the foreign malarias.

The intensity of infection in the individual mosquitoes, viz, the number of oocysts per infected gut was 148.

Malarias from each major area tested, viz South Pacific, Caribbean, Mediterranean, and China Burma India theater, produced heavy infections in the mosquitoes.

The infected mosquitoes usually showed comparable densities of

With Australian mosquitoes Mackerras and Roberts (1947) found that *A p farauti*, *annulipes*, *amictus*, and *bancrofti* showed similarly high susceptibility to New Guinea *P vivax* and that *stigmaticus* had a slightly lower susceptibility. *A p punctulatus* and *A longirostus* from New Guinea tested against the same strains of *vivax* showed a high susceptibility for the former and a lower susceptibility for the *A longirostus*.

#### DISCUSSION OF "P vivax"

It appears that exotic strains of *vivax* from various parts of the world demonstrate a high infectivity to certain important anopheline vectors, viz, *A quadrimaculatus*, *A m freeborni* (United States), *A p farauti* (Australia), and *A m atroparvus* (England). An important vector showing an exception was *A albimanus* from the Caribbean and United States.

However, different species of anophelines from the United States, viz, *A m freeborni*, *A quadrimaculatus*, *A p pseudopunctipennis* and *A albimanus*, showed a wide variation in susceptibility to any one strain of exotic malaria, viz, *A quadrimaculatus* showed a consistently high susceptibility, while *A albimanus* had a consistently low susceptibility.

A theoretical numerical evaluation of the various species tested shown in table 1. *A quadrimaculatus*, as the control species, is given an arbitrary value of 100, and the others evaluated on a comparative basis. From this table, it is seen that the various species of mosquito can vary widely in their susceptibility to any one strain of *vivax* malaria under identical experimental conditions. The variation in susceptibility of various mosquitoes to one strain of *vivax* appears to be much greater than the variation in the infectivity of one strain of *vivax* to various important species of mosquitoes.

#### OBSERVATION ON 'P falciparum'

In over 1,000 troops returning to this country with foreign malaria, Young et al (1948) found only 8 cases of *P falciparum*. One from the Mediterranean and one from Guadalcanal infected mosquitoes, lightly, but the data were insufficient for comparison.

Data by other workers are shown in table 2. Boyd et al (1938) found that Nearctic *A quadrimaculatus* and *A pseudopunctipennis* showed a high susceptibility to *P falciparum* from the Nearctic and the Neotropical regions. *A albimanus* from the tropical region was highly susceptible to *falciparum* from the same region but much less susceptible to that species from the Neotropical region. Mexican *A pseudopunctipennis* was inferior to *A quadrimaculatus* in susceptibility to Mexican and Nearctic *falciparum* and Earle, 1939).

Mackerras and Roberts (1947) found that *P falciparum* from

# 3 MALARIA

Table 1.—Infectivity of *P. vivax* to certain anopheline mosquitoes. The control and test mosquitoes were fed simultaneously on the same patient.

| <p> <i>virax</i> to certain anopheline mosquitoes. The control and test mosquitoes were fed simultaneously on the same patient </p> |                             |                  |                       |                  |                                    |                           |   |  |  |
|---|-----------------------------|------------------|-----------------------|------------------|------------------------------------|---------------------------|---|--|--|
| Origin of plasmodia   | Control anophelines         |                  | Test anophelines      |                  |                                    | Origin of test mosquitoes | Reference   |  |  |
|   | Dissected                   | Percent infected | Dissected             | Percent infected | Theoretical ratio when equal = 100 |                           |   |  |  |
| New Guinea  | <i>A. quadrimaculatus</i>   |                  |                       |                  |                                    | United States             | Young and Burgen, 1945  |  |  |
| Solomon Islands   | 434                         | 80.4             | <i>A. m. farborni</i> |                  |                                    |                           |   |  |  |
| New Hebrides  | 227                         | 36.7             | 447                   | 61.3             | 123                                |                           |   |  |  |
| Medan, Sumatra  | 61                          | 44.9             | 368                   | 32.2             | 107                                |                           |   |  |  |
| India   | 48                          | 43.6             | 49                    | 9.0              | 375                                |                           |   |  |  |
| China Burma India   | 47                          | 21.3             | 46                    | 32.1             | 36                                 |                           |   |  |  |
| Trinidad  | 19                          | 42.3             | 33                    | 64.8             | 26.5                               |                           |   |  |  |
| Total foreign   | 31                          | 0.0              | 77                    | 33.1             | 33                                 |                           |   |  |  |
|   | 521                         | 41.7             | 72                    | 34.7             |                                    |                           |   |  |  |
|   |                             |                  | 870                   | 32.7             | 110                                |                           |   |  |  |
|   | <i>A. punctipennis</i>      |                  |                       |                  |                                    | do                        |   |  |  |
|   | <i>A. quadrimaculatus</i>   |                  |                       |                  |                                    | do                        |   |  |  |
|   | 83                          | 91.8             | 48                    | 82.4             | 107                                | United States             | Young et al., 1945<br>Doyle and Kitchen, 1928<br>Doyle et al., 1938 |  |  |
|   | 173                         | 82.0             | 94                    | 63.2             | 63                                 |                           |   |  |  |
|   | 110                         | 80.9             | 39                    | 41.0             | 81                                 |                           |   |  |  |
|   | <i>A. parvipunctipennis</i> |                  |                       |                  |                                    | do                        |   |  |  |
|   | <i>A. quadrimaculatus</i>   |                  |                       |                  |                                    | do                        |   |  |  |
|   | 317                         | 80.9             | 61                    | 31.6             | 41                                 |                           |   |  |  |
|   | <i>A. quadrimaculatus</i>   |                  |                       |                  |                                    |                           |   |  |  |
|   | 44                          | 64.8             | 373                   | 1.8              | 7                                  | United States             | Young et al., 1945  |  |  |
|   | 30                          | 90.7             | 52                    | 0.0              | 0                                  |                           |   |  |  |
|   | 67                          | 67.4             | 21                    | 64.6             | 81                                 |                           |   |  |  |
|   | 63                          | 77.4             | 64                    | 1.8              | 82                                 |                           |   |  |  |
|   | 90                          | 72.2             | 64                    | 1.8              | 7                                  |                           |   |  |  |
|   | 60                          | 67.9             | 39                    | 2.6              | 6                                  |                           |   |  |  |
| <p> <i>A. quadrimaculatus</i> and <i>A. m. farborni</i> shown by Young and Burgen (1945) are broken by arms of the malaria. </p>    |                             |                  |                       |                  |                                    |                           |   |  |  |
| <p> <i>A. quadrimaculatus</i> and <i>A. m. farborni</i> shown by Young and Burgen (1945) are broken </p>                            |                             |                  |                       |                  |                                    |                           |   |  |  |
| <p> <i>A. quadrimaculatus</i> and <i>A. m. farborni</i> shown by Young and Burgen (1945) are broken </p>                            |                             |                  |                       |                  |                                    |                           |   |  |  |
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| <p> <i>A. quadrimaculatus</i> and <i>A. m. farborni</i> shown by Young and Burgen (1945) are broken </p>                            |                             |                  |                       |                  |                                    |                           |   |  |  |
| <p> <i>A. quadrimaculatus</i> and <i>A. m. farborni</i> shown by Young and Burgen (1945) are broken </p>                            |                             |                  |                       |                  |                                    |                           |   |  |  |

The gross data for *A. quadrimaculatus* and *A. m. fitchii* shown by Young and Burgen (1945) are broken down in this table by areas of origin of the plasmodia.

is infective to *A. quadrimaculatus* and that *A. punctipennis* is more favorable to *A. quadrimaculatus* as a host for indigenous and exotic (Cuban) *P. vivax* malarial. They found that *A. albimanus* from Cuba was less susceptible to an exotic *P. vivax* than to indigenous strains. It is similar to our experience that *albimanus* from this country is quite refractory to exotic *P. vivax* malarial and certain anophelines. In other countries, similarly favorable host-parasite relationships have been shown between exotic malarial and certain anophelines. Rute (1940) states that no difficulty was experienced in infecting *A. a'roparicus* with six different strains of *P. vivax* from the temperate zone and the tropics.

With Australian mosquitoes Mackerras and Roberts (1947) found that *A. p. farauti*, *annulipes*, *amictus*, and *bancrofti* showed similarly high susceptibility to New Guinea *P. vivax* and that *stigmaticus* had a slightly lower susceptibility. *A. p. punctulatus* and *A. longirostris* from New Guinea tested against the same strains of *vivax* showed a high susceptibility for the former and a lower susceptibility for the *A. longirostris*.

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Mackerras and Roberts (1947) found that *P. falciparum* from

Guinea and the Solomon Islands infected the Australian *A. annulipes*, *A. a. amictus*, and *A. hilli*. In England, Shute (1940) infected *A. m. atroparvus* with *parum* from Italy, Sardinia, and Rumania, but failed with mosquitoes from the tropics, so that comparative susceptibility were not known. However, the patients had many gametocytes flagellation was observed, and some ookinetes were seen.

TABLE 2.—Infectivity of *P. falciparum* to certain anopheline mosquitoes from various reports

| Susceptibility of <i>P. falciparum</i> to certain anopheline mosquitoes from various reports |                           |                  |                              |                  |                                     |  |  |              |                              |
|--|---------------------------|------------------|------------------------------|------------------|-------------------------------------|--|--|--------------|------------------------------|
| Origin of plasmodia  | Control anophelines       |                  | Test anophelines             |                  |                                     | Origin of test mosquitoes                              | Reference  |              |                              |
|  | Dissected                 | Percent infected | Dissected                    | Percent infected | Theoretical value when quadr. = 100 |  |  |              |                              |
| United States<br>Panama<br>Mexico<br>Cuba<br>Do<br>United States<br>Do                       | <i>A. quadrimaculatus</i> |                  | <i>A. edwardsii</i>          |                  |                                     | Panama<br>do<br>do<br>Cuba<br>Panama<br>Cuba<br>Panama | Boyd and Jobbins, 1942.<br>Do.<br>Do.<br>Boyd et al., 1934.<br>Do.<br>Do.<br>Do. |              |                              |
|  | 45                        | 48.9             | 40                           | 7.5              | 13                                  |  |  |              |                              |
|  | 91                        | 17.6             | 103                          | 12.6             | 7                                   |  |  |              |                              |
|  | 35                        | 5.1              | 37                           | 13.5             | 24                                  |  |  |              |                              |
|  | 65                        | 30.6             | 61                           | 24.6             | 44                                  |  |  |              |                              |
|  | 100                       | 61.6             | 100                          | 4.2              | 7                                   |  |  |              |                              |
|  | 130                       | 42.3             | 33                           | 6.1              | 10                                  |  |  |              |                              |
|  | 20                        | 52.9             |                              |                  |                                     |  |  |              |                              |
|  | <i>A. quadrimaculatus</i> |                  | <i>A. pseudopunctipennis</i> |                  |                                     |  |  | Mexico<br>do | Boyd and Earle, 1939.<br>Do. |
|  | 44                        | 61.2             | 28                           | 7.1              | 16                                  |  |  |              |                              |
| Do<br>Mexico   | 42                        | 7.6              | 74                           | 4.1              | 5                                   | Cuba<br>United States                                  | Boyd et al., 1935.<br>Boyd and Kitchen, 1936.                                    |              |                              |
|  | <i>A. quadrimaculatus</i> |                  | <i>A. punctipennis</i>       |                  |                                     |  |  |              |                              |
| Cuba<br>United States  | 91                        | 51.6             | 47                           | 16.7             | 32                                  | United States<br>do                                    | Boyd et al., 1935.<br>Boyd and Kitchen, 1936.                                    |              |                              |
|  | 201                       | 65.6             | 5                            | 52.9             | 81                                  |  |  |              |                              |
| India<br>West Africa<br>East Africa<br>Italy<br>Sardinia<br>Rumania                          | <i>A. m. atroparvus</i>   |                  | <i>A. m. atroparvus</i>      |                  |                                     | England<br>do<br>do<br>do<br>do                        | Shute, 1940.<br>Do.<br>Do.<br>Do.<br>Do.   |              |                              |
|  | No control                | 100 ± 21.9 ± 7   | 0.0                          | 0.0              | ---                                 |  |  |              |                              |
| New Guinea<br>Solomon Islands  | do                        | ---              | do                           | 0.0              | ---                                 | Australia  | Mackerras and Roberts, 1944  |              |                              |
|  | do                        | ---              | do                           | 0.0              | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
| do   | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |
|  | do                        | ---              | do                           | +                | ---                                 |  |  |              |                              |



DISCUSSION OF '*P. falciparum*'

Important vectors from four regions, viz, United States, Caribbean Australia, and England, have been tested against *P. falciparum*. Two important vectors, *A. quadrimaculatus* and *A. m. farauti*, have shown a high susceptibility to native and exotic strains of *P. falciparum*. *A. albimanus*, however, is highly susceptible to native strains but less so to exotic strains. There are indications that *A. m. atroparvus* is susceptible to *P. falciparum* from the Palearctic region but not to the parasites from tropical Africa and India.

## GENERAL DISCUSSION

Boyd et al (1938), have suggested that there may exist between particular strains of malaria parasites and their insect vectors a very high degree of local adaptation which, under certain conditions, may conceivably be a natural barrier to the extension of the range of a given strain of the parasite.

*P. falciparum* showed a wide range of adaptability in this respect. New Guinea parasites infected Australian mosquitoes well, Caribbean parasites infected American *A. quadrimaculatus*, and European parasites infected British *A. m. atroparvus*. American parasites infected Caribbean *A. albimanus* poorly, West African and Indian parasites did not infect British *A. m. maculipennis*.

*P. vivax*, on the other hand, has shown a more uniform infectivity to important vectors. Australian, American, and British mosquitoes apparently were highly susceptible to exotic strains of *P. vivax*. So far, the major exception is the refractoriness shown by Caribbean and American *A. albimanus* to exotic *P. vivax* malarial parasites. However, the exotic malarial parasites did not infect equally well all of American species of mosquitoes. The most dangerous carriers of native malarial parasites were also the most susceptible to the exotic *P. vivax* strains.

The results so far indicate a varying susceptibility among anopheline mosquitoes to malaria. Because of the few and widely separated places where the work has been pursued, no definite broad patterns can be laid down. In view of the rapidity with which human carriers and mosquito vectors of malaria can be transported to different countries, more information is needed about the definite host-parasite relationships in malaria. Not only is such knowledge desirable for new mosquito-parasite combinations, but also for many of the vector-parasite combinations now existing.

As a result of experimental work just before and during War II, the knowledge of host-parasite relationships has been enlarged somewhat, but the amount of ignorance still remaining is great. We know from epidemiological experience after the last two Wars especially, that malaria can be introduced into normally free areas. Much more knowledge on the adaptability of malarial parasites to important host vectors is needed to be able to pre-

V MALARIA

V MALARIA

In England, Shute (1940) infected *A. m. atroparvus* with *P. falciparum* from Italy, Sardinia, and Rumania, but failed with mosquitoes from the tropics, so that comparative susceptibility was not known. However, the patients had many gametocytes and flagellation was observed, and some ookinetes were seen.

TABLE 2.—*Infectivity of P. falciparum to certain anopheline mosquitoes from various reports*

| Origin of plasmodia | Control anophelis         |                  | Test anophelis  |                  |                                   | Origin of test mosquitoes | Reference  |
|---------------------|---------------------------|------------------|---|------------------|-----------------------------------|---------------------------|--|
|                     | Dissected                 | Percent infected | Dissected   | Percent infected | Theoretical value when quad = 100 |                           |  |
| United States       | <i>A. quadrimaculatus</i> |                  | <i>A. albimanus</i>   |                  |                                   | Panama                    | Boyd and Johnson 1940<br>Do<br>Do<br>Do<br>Boyd et al 1933<br>Do<br>Do<br>Do |
| Panama              | 45                        | 43.9             | 40  | 7.6              | 15                                |                           |  |
| Mexico              | 41                        | 37.6             | 103   | 13.6             | 77                                |                           |  |
| Cuba                | 25                        | 67.1             | 37  | 13.6             | 24                                |                           |  |
| Do                  | 45                        | 50.8             | 61  | 24.6             | 48                                |                           |  |
| United States       | 170                       | 65.0             | 100   | 47.0             | 72                                |                           |  |
| Do                  | 130                       | 62.3             | 72  | 4.2              | 7                                 | Mexico                    | Boyd and Earle 1939<br>Do<br><br>Boyd et al 1932a<br>Boyd and Kitchen 1936   |
| Do                  | 29                        | 59.0             | 53  | 6.1              | 10                                |                           |  |
| Do                  | <i>A. quadrimaculatus</i> |                  | <i>A. pseudopunctipennis</i>  |                  |                                   |                           |  |
| Mexico              | 44                        | 43.2             | 25  | 7.1              | 16                                |                           |  |
| Do                  | 42                        | 78.6             | 24  | 4.1              | 8                                 |                           |  |
| Cuba                | <i>A. quadrimaculatus</i> |                  | <i>A. punctipennis</i>  |                  |                                   |                           |  |
| United States       | 81                        | 81.8             | 47  | 16.7             | 32                                | United States             | Boyd et al 1932a<br>Boyd and Kitchen 1936                                    |
| Do                  | 201                       | 85.6             | 87  | 52.8             | 81                                |                           |  |
| India               | <i>A. m. atroparvus</i>   |                  | <i>A. m. atroparvus</i>   |                  |                                   |                           |  |
| West Africa         | No control                | 100±             | 0.0   |                  |                                   |                           |  |
| East Africa         | do                        | 310±             | 0.0   |                  |                                   |                           |  |
| Italy               | do                        | (?)              | 0.0   |                  |                                   |                           |  |
| Sardinia            | do                        |                  | +   |                  |                                   | England                   | Shute 1940<br>Do<br>Do<br>Do<br>Do<br>Do                                     |
| Rumania             | do                        |                  | +   |                  |                                   |                           |  |
| Do                  | do                        |                  | +   |                  |                                   |                           |  |
| Do                  | do                        |                  | +   |                  |                                   |                           |  |
| Do                  | do                        |                  | +   |                  |                                   |                           |  |
| Do                  | do                        |                  | +   |                  |                                   |                           |  |
| New Guinea          | <i>A. p. punctulatus</i>  |                  | <i>A. p. farauti</i><br><i>A. annulipes</i><br><i>A. e. annulipes</i> |                  |                                   | Australia                 | MacKerras and Roberts, 1947  |
| Solomon Islands     |                           |                  |   |                  |                                   |                           |  |

† = Infected. No percentages stated.  
± = More or less.

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### ABSTRACT OF DISCUSSION

SIR GORDON COVELL (United Kingdom) I would like to make one comment on this paper. In the last 3 or 4 weeks at Horton, England, we have been feeding three species of anopheles on the same human carrier of a *falciparum* strain from West Africa. We fed these three species at the same time on the same patient. One, *A. maculipennis* var. *atroparvus*, produced no infection at all on any of those fed. This confirms the experiments done before by Shute, to which Dr Young alluded just now. *A. quadrimaculatus* got the largest proportion of infections, about 80 percent, but the infections were very scanty indeed. With *A. stephensi*, the Indian strain, the same proportion of mosquitoes became infected but the infections were very much heavier. A large proportion of them had more than 50 oocysts on each stomach.

possibly prevent, the establishment of new strains of malaria in various parts of the world

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The mosquito used as a standard should be preferably an important

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tible provided that normal infections develop in nearly all of the specimens that 100 or more

### SUMMARY AND CONCLUSIONS

As a result of studies just before and during World War II, the knowledge of the ability of anophelines to transmit exotic malarial was increased

In case 1 of 2

to

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that malaria vectors and in general appeared to show a more selective vector parasite adaptation

There is still much essential information lacking on the adaptability of malaria parasites to vectors of different regions. Such knowledge would be of particular importance in the event of large scale migrations of peoples between or from malarious areas

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Boyd M F

temperate zones of Chungking, *minimus* passes the winter in larval stages

A few experiments on longevity of adults were attempted but gave no conclusive data. Some mosquitoes of this species lived in cages for about 15 days, but their life in nature is presumably longer. On the basis of experimental control work it is believed that the effective flight range is not more than half a mile.

**Feeding habits and blood preference**—Thomson (1911) in Assam reported upon results during night catching of *minimus* in houses, stating that about 90 percent of blood feeding took place after mid night. According to our observations with buffalo and horses as bait, *minimus* fed intermittently from 8 p m until 4 a m, mostly during the hours from 10 p m to 2 a m. After feeding, the majority of adults remained in the cowshed during the following day, while some of them left the place at 5-6 a m. Those leaving in the early morning probably move to other cowsheds or houses for resting in the daytime. Thus DDT residual spray would be useful for killing them in the buildings where they remain after feeding, at least until the following early morning. Regarding its host preference, *minimus* has usually been regarded as anthropophilic. However, the results of precipitin tests of 1,665 *minimus* caught in houses and cowsheds in Yunnan showed that it had no special preference and apparently it is a matter of availability. Of the number caught in houses 67 percent had human blood against 42 percent positive for cow blood, 1 percent and 85 percent were the relative percentages of specimen taken from cowsheds. In Chungking, on the other hand, of 82 *minimus* caught in cowsheds 7 percent had human blood against 6 percent with cow blood.

**Egg laying and life cycle**—A *minimus* will lay eggs on the third to fifth nights after taking blood. One blood meal is required for the maturation of each batch of eggs. They lay eggs at night, most before midnight. The number of eggs laid by a female after a blood meal varied from 83 to 168, with an average of 120. The total production of a female during her lifetime has not been determined. The eggs are laid along the margins of slow flowing water. Larvae live in clean, sunlit, slowly running water, such as streams, irrigation channels, ditches, and springs. Occasionally they are found in fields, ground holes, and rock holes. The larvae scatter in their breeding places but seem to concentrate in masses in certain spots of stream during hibernation. Under favorable conditions the duration egg to adult is about 16 days, i. e., from egg to first instar, 2 days; second instar, 3 days; to third instar, 2 days; to fourth instar, 3 days; to pupa, 4 days; and to adult, 2 days.

These observations suggest that *minimus* is a good malaria vector because of its domestic habits, seasonal density, length of life, and access to human blood. Dissections of 27,603 *minimus* (Sweet 1912, and later work) at Chefang on the Yunnan Burma Road

## THE BIONOMICS OF TWO IMPORTANT MALARIA VECTORS IN CHINA

Y CHOW, *Malaria Laboratory, National Institute of Health,  
Ministry of Health, Nanking, China*

Up to the present, 38 species and varieties of anopheline mosquitoes have been reported in China and their distribution in China.

In 1941, Sweet et al., 1942, reported in an appendix. Two species, *Anopheles minimus* and *A. hyrcanus sinensis*, are considered of greatest importance in malaria transmission in the hilly regions of South China and in the plains of Central China, respectively. *A. peyponensis candidiensis* may be of secondary importance as a malaria vector in the hilly region of South China, *A. pattoni* in the hilly regions of North China, *A. maculipennis* (? *atroparvus*) in Heilungkiang (Manchuria), and *A. sacharovi* in the plains of North China.

The present paper reports on the bionomics of the two most important vectors, *A. minimus* and *A. hyrcanus sinensis*.

### BIONOMICS OF "ANOPHELES MINIMUS"

This domestic species prefers to rest by daytime in dark shelters in human dwellings; they are usually found inside and behind the

species was observed entirely in cowsheds in Chungking.

It is the predominant species in South China. There are two peaks of adult density in a year: one in September and October, when the temperature becomes cooler, and another in May and June, when the temperature becomes warmer. The numbers of adults are more numerous in the autumn than in the spring, and they fall during the winter from their

activity in maintaining a low density of adults during August. In September the light autumn rains and other climatic conditions favor an increase of *minimus* density, so this species reaches its climax in October-November. No hibernation occurs in subtropical regions such as Southwest Yunnan and South Taiwan (Formosa), while in the

females laid eggs throughout the night. In field and laboratory experiments it has been demonstrated that *sinensis* can still lay eggs on the water when the rice plants are as high as 5 feet and very dense. Larvae occur in both still and running waters, especially in rice fields. The life cycle from laying eggs to imago is fully covered with vegetation like *Azolla* and *Lemna*, reports of 0.1-0.3 months.

The natural host is man, and in some cases reports of 0.1-0.3 percent in Central China), and in some cases it shows a preference for animal blood. However, *sinensis* appears to be the chief, if not the only, malaria vector in the plains of Central China. Whether there are different races of this species is still unknown.

### SUMMARY

Thirty eight species of anopheles have been reported from China. Among them *A. minimus* and *A. hyrcanus sinensis* are the two species of greatest importance in malaria transmission in South China and Central China respectively. *A. minimus* is domestic and is abundant, with two density peaks in the year. It feeds on both human and cow blood throughout the night. *A. hyrcanus sinensis* is the predominant species in Central China and prefers cowsheds as its daytime resting place. Its greatest density is in May. Although this species prefers animal blood, it feeds also on humans, and most of its feeding occurs before midnight.

### APPENDIX

Anopheles species occurring and their known geographical distribution in China

- A. aconitus* Dönitz—Hainan Is. and Yunnan
- A. aitkeni* James—Chekiang and Kwangsi
- A. aitkeni bengalensis* Puri—S. China
- A. annandalei interruptus* Puri—Yunnan
- A. annularis* V. d. Wulp—S. China
- A. barbirostris* V. d. Wulp—Hainan Is. Kwangtung, Szechuan and Yunnan.
- A. barbumbrosus* Strickland & Chowdhury—Taiwan (Formosa)
- A. culicifacies* Giles—Yunnan
- A. flutator* James—Fukien, Kwangtung, Szechuan, Taiwan and Yunnan
- A. gigas baileyi* Edw.—Kweichow, Szechuan, Taiwan, Tibet and Yunnan
- A. gigas simlensis* James—Kweichow and Tibet
- A. hyrcanus nigerrimus* Giles—Hainan and Yunnan
- A. hyrcanus sinensis* Wiedemann—Cosmopolitan in China
- A. insulæstorum* (Swellengrebel & S. de Graaf)—Taiwan
- A. jamaicae* Theobald—Hainan and Yunnan
- A. jeyporiensis candidiensis* Koldzumbe—S. China
- A. laricari* James—Kwangtung, Kwangsi and Yunnan
- A. lochi* Dönitz—Hainan, Kwangtung, Kwangsi and Yunnan
- A. loreus* Yamada & Watanabe—Chekiang
- A. kweiyangensis* Yao & Wu—Kweichow
- A. leucosphyrus* Dönitz—Hainan, Taiwan and Yunnan
- A. lindesayi japonicus* Yamada—Hokkaido, Shantung, Szechuan and Sikan
- A. lindesayi lindesayi* Giles—Central and South China

a monthly natural infection rate of malaria parasites to be 1 per cent varying from 0.5 percent (in January and February) to 3.7 per cent (in July through November). It can transmit malaria throughout the whole year in that region.

### BIOLOGICS OF "*A. HYRCANUS SINENSIS*"

This species predominates in the plains of Central China and seems to be the only anopheline mosquito found in certain areas like Nanking and Shanghai. Its greatest adult density is in May. According to climatic conditions, the main factor which influences the density of this species is the rice culture. In subtropical regions it occurs throughout the year, but in the temperate zone it hibernates as adult and larva. It prefers cowsheds for daytime resting places. However, the mosquitoes in a cowshed will decrease to a very small number on the following day after the removal of cows from the building.

**Feeding habits and blood preference.**—The mosquitoes start to move out from the cowsheds, where they have spent the whole day, around 5 p. m., and movement increases markedly at 6 p. m., or just before sunset. It decreases after 6.30 p. m. and no activity has been observed from 8 a. m. to 4 p. m. The mosquitoes flying from the cowshed in the evening are mostly unfed ones and are seeking blood. Those leaving the cowsheds in the early morning are mostly fully fed and are seeking suitable daytime resting places. The fed ones with fully developed ova flying from cowsheds in the evening are undoubtedly seeking places for egg deposition. However, many young females, fully fed, with undeveloped ova, also leave at this time. The purpose of this flight of young, fully fed females is not apparent. Unfed females attack buffalo in large numbers by 6.30 p. m., just after sunset. Their number decreases from then on, but feeding increases again suddenly around 6 a. m., just before sunrise. The number feeding during the period before midnight is distinctly higher than after midnight.

This species has been usually regarded as zoophilic. However, in reciprocal tests of 505 specimens from Yunnan human blood was observed in 55 percent from houses and 45 percent from cowsheds. Tounanoff and Hu (1935) also reported from Shanghai that 165 *sinensis* of human blood of 172 specimens tested, being over 96 percent, but of those caught in cowsheds had bovine blood. In Chungking we had that *sinensis* had cow blood in 95 percent of the specimens from cowsheds and 83 percent of those from houses among 800 specimens. It is interesting to note that even though hundreds of pigs existed in Nan only 1 percent were positive for pig blood. The number of eggs laid by a female during laying and life cycle.—The number of eggs laid by a female from 115 to 255 per batch with an average of 150. The eggs laid on the second to fourth nights after taking a blood meal seem to be enough for the maturation of ova. The



# THE CONTROL OF BROMELIAD MALARIA IN TRINIDAD, BRITISH WEST INDIES

Dr H. P. S. GILLETTE, *Malariaologist, Malaria Division, Health Department, Trinidad and Tobago*

Downs and Pittendrigh use the term 'bromeliad malaria' to cover cases where the vector is bromelicolous, as it emphasizes the unity of these cases in the uniqueness of the control problems that are presented. An important phase of the present work of the Malaria Division of the Health Department of Trinidad and Tobago is the control of this bromeliad malaria.

An excellent and adequate historical résumé of the investigations incriminating members of the subgenus *Kerteszia* as vectors of malaria is to be found in "A Malaria Survey of Trinidad and Tobago", and "Bromeliad Malaria in Trinidad". Although I. W. Ulrich, a Trinidad entomologist of considerable local renown, first thought that *A. bellator* (D. and K.) might be the cause of malaria upon the cacao and other plantations in Trinidad, it was left to Rozeboom and Laird and Downs, Gillette and Shannon finally to incriminate this mosquito.

One of the most striking features of the bromeliad malaria situation in Trinidad is the overwhelming importance of *A. bellator* as the vector species. This mosquito is one of at least four species of the subgenus *Kerteszia* known to occur in Trinidad. *A. anoplus* and *A. (Kerteszia) sp.* are rare, but *A. homunculus* is also common in the forested areas. The cacao estates in Trinidad are the forest type that differ markedly from the natural seasonal and rain forests of the island in their open structure, brought about by the wide and

which permits  
o the very tops  
ultivated cacao

to *A. homunculus* but are  
experimental work has shown a  
ution on the forest of these  
climatic conditions ranging  
de of the forest floor to the

dryness of the forest canopy to the humid conditions of the rain forest. The cacao tree is itself of the lower strata of Rain Forest, re  
nor

trees are planted throughout the cacao plantation, giving medium shade over the entire plantation. Due to the construction of the cacao forest in which the deciduous *immortelle* is so regularly

# V. MALARIA

- A. ludlowi* Theobald—Hainan and Taiwan
- A. maculatus* Theobald—S China
- A. maculipennis* Meigen—Heilungkiang (Manchuria)
- A. minimus* Theobald—South of 30° N Lat
- A. pattoni* Christophers—Honn, Hopei, Shantung, Sikang, and Sz
- A. philippinensis* Ludlow—Hainan and Yunnan
- A. sacharovi* Fuhr—Sinkiang
- A. sinensis* Yamada—Chekiang
- A. sinonoides* Ho—Hainan Is
- A. splendidus* Koidzumii—S China
- A. stephensi* Liston—Yunnan
- A. subpictus* subpictus Ludlow—Hainan and Taiwan
- A. subpictus* subpictus Grassi—Kwangtung and Yunnan
- A. subpictus* subpictus Grassi—Kwangtung, Taiwan and Yunnan
- A. tessellatus* Theobald—Hainan, Kwangtung, Kwangsi, and Yunnan
- A. vagus* Donitz—Hainan, Kwangtung, Kwangsi, and Yunnan

## ACKNOWLEDGMENT

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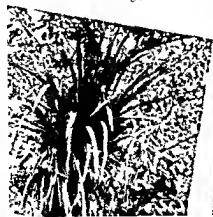




A *Gravisia aquilega* The major host plant of  
A bellator



Section *Gravisia aquilega*



sulphate

ma



F The gypsy moth sprayer at work



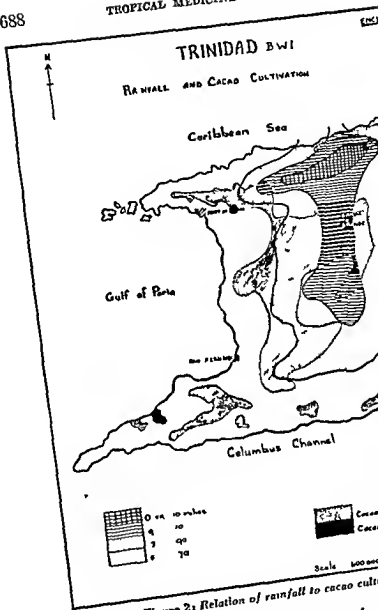


Figure 2: Relation of rainfall to cacao cult

*A bellator* occasionally enters dwellings to feed taken in appreciable numbers within the house doors, but leaves for forest cover immediately. The flight range of this mosquito is very limited usually most half a mile.

The control of bromeliad malaria is intimately connected with the cacao industry of the Colony. Since this malaria is invariably due to cultural practices, an exter

arranged, the microclimate suitable for *A. bellator* is found at the ground thru in natural forests and will even approach general level on the edges of the clearings in which villages are found. The general conditions so created have also proved ideal for the growth and spread of the large tank bromeliads, and these are present in the immortelles in densities that are found nowhere in the native vegetation types of Trinidad. The epiphytic bromeliads in the common forest types of Trinidad fall into three more or less distinct ecological categories:

- (1) Bromeliads in the society of exposure epiphytes
- (2) Bromeliads in the society of sun epiphytes.
- (3) Bromeliads in the society of shade epiphytes.

We are mostly concerned with this second group of bromeliads in the society of sun epiphytes. Here are found *Gravisa aquilega*, *Hohenbergia stellata*, and *Waltmackia lingulata*, the principal host plants of *A. bellator* and the plants with which our control measures have been mainly concerned (Fig 1).

Primary host plants support *A. bellator* breeding all the year round. They hold on an average one half to 1 litre of water, and it is this group of plants, therefore, that is responsible for carrying the mosquito population through dry weather.

Secondary host plants support considerably less breeding than primary hosts and are only important during the rains. The chief of these are *Vriesia Amazonica*, *V. macrostachya*, and *Aferobromelia splutgerborn*.

Another striking aspect of the epidemiological picture of bromeliad malaria in Trinidad is that, although the cultivation of cacao extends in a belt along the foothills to the southern portion of the island, and although the host plants of *A. bellator* are present in great profusion in the immortelles throughout these plantations, the mosquito does not occur everywhere with equal density. Rainfall is the limiting factor. Only in the areas of heavy rainfall does *A. bellator* maintain itself in continuously high densities. These densities progressively fall off as one passes into areas of lower precipitation. This is not strange if we use the distribution of *bellator* throughout the micro climatic cline of the forest profile as a guide to its distribution through out a similar macro climatic cline. The absence of the species in the dry Southwest can, therefore, be readily understood (Fig 2).

Another feature of *A. bellator* (and *A. homunculus*) that must be remembered in order to appreciate its importance in the transmission of malaria, is the fact that under forest cover it is active all through the day and laborers are liable to be bitten throughout the day. In fact, *bellator* bites readily at noon. There are, of course, peaks of activity at dusk, when an exceptionally heavy flight occurs with amazing regularity, and at dawn, when the peak is not as marked as at

and the cost would be prohibitive. Attention became  
 on the host plants themselves, and here  
 manual removal of

and yet now

The manual removal of  
 serious drawbacks apart from its high cost  
 has from 30 to 100 major host plants firmly attached to it  
 major host plants need be dealt with, and labourers can readily be  
 taught to recognize these plants and thus practice species control. The  
 immortelle tree is both difficult and dangerous to climb. It is covered  
 with large thorns and cannot be readily grasped. Rope cannot always  
 be used, for the wood is brittle and often the lower branches are rotten  
 and fungus infected and break away easily. Snakes, scorpions, and  
 spiders find harbourage in the luxuriant growth of epiphytes with  
 which the tree is adorned, and it is usual to find several large nests  
 of ferocious biting ants on a single tree. The economics of manual  
 removal can be best gaged by a few pertinent facts. The acreage  
 under cacao where bromeliad malaria is the main cause of illness  
 nearly 50,000 acres out of the 130,000 cultivated acres in the entire  
 colony. There are, on the average, 30 immortelles per acre of cacao.  
 The average cost of clearing a tree of its specific host plants is  
 which neither the economy of the crop nor Government can support.

Nevertheless, manual removal has its place in the control of bromeliad malaria as a necessary ancillary method in a sprayed area to  
 with the occasional missed tree as well as in difficult types of terrain  
 which do not lend themselves to spraying.

Cohn Pittendrigh, seeking for an economic method for the extermination of bromeliads, was considerably attracted by the unique physiology of these plants and was convinced that this was the obvious spot in their economy. The bromeliad, a true epiphyte, uses its only as a mechanical means of attachment to the tree on which it grows. The absorption of nutriment for the growth and development of the plant takes place through specialized absorbing tissue in the leaves which form the water holding, or tank, part of the plant. It seemed possible to apply a herbicide in the tank of the plant which would be absorbed and which would also not be damaging to the immortelle or to the cacao tree beneath it. After experimental trials with several herbicides, copper sulphate was found to give the most promise, and a field station for large scale trials was set up in the heart of a cacao district with very heavy precipitation. The results of several issues to be determined.

- (1) The possibility of copper damage to cacao or other cover crops, like peas, dasheen (ground provisions).
- (2) The development of efficient operating methods.

such practices was made with a view to their modification. In the

plantations on the Paria peninsula of Venezuela are shaded by *Tecoma pentaphylla* (pink pou) which is nondeciduous and extremely leafy. Bromeliads do not grow to any extent on these trees, also abundant in Trinidad. A survey of trees that could be used for shade and yet not be suitable for bromeliads reveals that, generally, non-

ate the falling of heavy limbs and even trees on the cacao trees below. The immortal is very readily subject to a fungus infection (*Colos tobe struspora*) and, being rather brittle, can be thrown down by high winds. Three possibilities are stressed in our discussion of new and improved cultural practices:

(1) The use of an evergreen tree with a heavier canopy than the deciduous immortal used at present.

(2) The complete abandonment of shade trees and the adoption of a system of close planting with windbreaks.

(3) The rapid rotation of immortelles on existing estates so as not to allow individual trees sufficient time to become seriously infested with bromeliads.

The Department of Agriculture has been experimenting to find new shade trees for cacao, and Thorold, in a preliminary paper, reports that there is considerable promise of so doing.

The introduction of new cultural practices is always a matter of extreme difficulty. The prejudices of the peasant proprietors are not easily overcome. The Government of Trinidad and Tobago has launched a Cacao Rehabilitation Scheme, and some small effort is being made to encourage a new method of close planting by offering a higher subsidy than for rehabilitation on the old system.

In any case, such a method of control, though the ideal method, is an extremely long range project highly dependent on the economic stability of cacao in the world.

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Furthermore, the application of such larvicides would have to be done often, particularly





- (3) The adoption of either total spraying of all pines or selective spraying of host plants and the relative training of personnel for large scale field work.
- (4) The first issue was very readily answered, and within a short time it was obvious that copper sulphate in strengths from 0.25 to 1 would kill bromeliads but leave undamaged the cultivation.

The second issue was beset with grave difficulties. Eventually the United States Department of Agriculture selected as being the most suitable culture in the control of the gypsy moth (*Lymantria dispar*) the third issue of total versus selective spraying more or less answers itself. Nozzlemen are trained to recognize at a glance the many host plants of *A. bellator*.

In the training of personnel, considerable attention had to be paid to ground procedure. The method is as follows:

The area selected for control operations is first visited by a small unit which makes a survey of the state of the cacao and immortelles, their average number, amount of bromeliad infestation, nature of the terrain, and location of water supply.

This unit is followed by a work unit called the "trace cutting" unit. Cacao cultivation proceeds in unbroken continuity, and most of the crop is dealt with *in situ* and "headed" to a cacao house. A series of traces, cleared through the cultivation to an average width of 3 feet, is laid down in a pattern of cross and main lines, every effort being made to avoid damage to the cacao, immortelle, or other economic crops. The cross lines are the traces from which the actual spraying of trees is done. They are laid down in long straight lines, 100 feet apart and parallel to one another and to the rows in which the cacao trees are planted. At right angles to the cross lines and 200 to 400 feet apart, main lines are laid down along which the lengths of hose are set out. The distance between the cross lines has been set at 100 feet because immortelles are generally planted in 25 foot squares. It is therefore possible to spray two rows of immortelles on either side of a cross line. The cacao trees beneath these immortelles are sprayed at the same time. The workmen therefore leave behind them a swathe of well sprayed vegetation at least 50 feet deep on either side of these lines.

The distance between the main lines depends on the condition of the cultivation and the nature of the terrain. On cacao estates which are actively cultivated and well maintained and are situated on fairly level ground, main lines may be cut as far apart as 400 feet, thereby allowing work to be done at a faster rate. Where the cultivation is abandoned and overgrown with bush, and the ground is steep, broken, and obstructed with drains and fallen trees, main lines must sometimes be laid down as close as 100 feet to one another. The lines of the pattern, an independent line or one of the lines of the pattern, is found most convenient, connects the main lines with the spraying

## ABSTRACT OF DISCUSSION

Dr. MARIO PINOTTI (Brazil) I am on this platform for a double purpose First, to offer my congratulations to Dr Gillette for his achievements obtained in Trinidad in regard to the problem of bromeliad malaria Second, to submit a brief report on the problem of bromeliad malaria along the southern coastal regions of Brazil, a problem varying in aspects from that of Trinidad

(a) There are three verified species of the *Kerteszia* in southern Brazil, *bellator*, *cruzi*, and *homunculus* The *cruzi* and *homunculus* predominate in the dense, humid, and shady primary wooded sections, the *bellator* predominates in the open, rocky places and in secondary wooded areas, that is, those not so dense, humid, and shady

(b) In southern Brazil, the anophelines of the subgenus *Kerteszia* are characterized epidemiologically as showing strong domesticity (about 99 percent of the anophelines captured in dwellings), very high density, high anthropophilia (attacking man at any time during the day and night, inside as well as outside of the house), and the capacity to transmit inside and outside the domicile

(c) Our bromeliad malaria is of very high endemicity and is caused by the three species of *Plasmodium* *vuax*, *falciparum*, and *malariae*

(d) Our problem of bromeliad malaria is not only a problem of jungle malaria, but also an important problem of urban malaria, including cities of great economic social expansion, such as the larger industrial centers of the State of Santa Catarina

(e) We cannot use sulfate of copper in combatting the bromeliad as it was done in Trinidad for the following reasons

- 1 Very precipitous topography of the terrain
- 2 The greater part of our forests, of the primary type, being 20 to 40 meters in height and very dense

Besides being very expensive, as we would have to use very much more powerful equipment than that used in Trinidad, the method could not assure even a relative success due to the aspects of our forests

(f) Up to 1947 we used the following methods in our campaign I The manual destruction of the bromeliads, a very slow and actively expensive process due to the necessity of periodical inspection in the treated areas. With this method, destroying about 25,000,000 the bromeliads, we were able practically to free Florianopolis capital of the State of Santa Catarina, from malaria In this of 40,000 inhabitants, from 1944 to 1947, we were able to obtain a reduction of 90 percent in the general morbidity rate of malaria 96.5 percent in the transmission rate In 1948, up to the time coming here (May 8), the period of the annual recrudescence passed, there was not a single primary autochthonous case reported in that city

II Deforestation.—Seeking the most rapid and economic method we replaced the manual destruction of the bromeliads by

its environs, nearly 2,000 acres of cacao had to be attended to for the morement of the district, severely attenuated by the movement of to other centers of employment, was about 30,000. Two schools the town showed a spleen rate of 28 percent in 1945, whilst in the year three schools about one half mile from the center of the and more wholly situated amidst the plantations showed rates percent and 43 percent. By the end of 1947, nearly 1,500 acres cacao had been sprayed with 0.25 percent to 0.5 percent copper fate at a cost varying from \$16 to \$27 per acre. Within a week spraying, the effects were noticeable. There is first a yellowing, the leaves which rapidly progresses so that by the end of a month the plant has completely dried up. In many instances the plant loses firm attachment and droops from the tree, but more often than not remains in its fixed position and may not droop and fall off until months or more have elapsed. Control operations began from the center of the town and spread out toward the periphery, the ultimate aim being to spray a belt of at least one fourth mile around the built up area.

The reduction in spleen rates has been gratifying. The two schools in Sangre Grande proper showed a spleen rate of 5 percent in 1947, whilst the schools on the periphery, not yet sprayed, still show rates from 25 percent to 35 percent. The district medical officer also reports a considerable decrease in malaria morbidity in the control area. There can now be no doubt about the efficacy of spraying bromeliads. It is more than a temporary control, for trees on which bromeliads have been destroyed by the application of copper sulfate 4 years ago have shown no signs of reinfestation. The rate of regeneration, if regeneration is going to occur, is obviously extremely slow. The growth of seedlings is also extremely slow, and an area that has once been sprayed will not require attention, so far as bromeliad malaria is concerned, for at least 10 years.

#### SUMMARY

*A bellator*, a forest species, has definitely been incriminated by several workers as the vector of malaria in the high rainfall cacao-growing districts of the island of Trinidad. It has been shown that this species breeds exclusively in certain members of the Bromeliaceae, of which *Gratisea agulega* is the most important. The problems of efficient control are discussed, and, whilst a changed agricultural practice in the cultivation of cacao would be the ideal solution, it is not remotely practicable for several reasons. Annual removal of bromeliads has its place in specific circumstances. Spray killing of bromeliads by the application of copper sulfate is only economical but efficient and is the method of choice. Bromeliads killed by copper sulfate are extremely slow to regenerate, and application may cover a 10-year period.

# EXPERIMENTS ON CONTROL OF ANOPHELINE LARVAE AND MALARIA IN RICE GROWING REGIONS OF PORTUGAL

F J C CAMBOURNAC, *Director of the Instituto de Malariaologia, Aguas de Moura, Portugal, Professor at the Institute of Tropical Medicine Lisbon* and A CRAGLIA DA FONSECA, *from the Estacao Anti Sero-natica Benavente, Portugal*

Malaria in Portugal occurs chiefly in the alluvial valleys of the rivers Sado, Tagus, and Mondego and their tributaries where rice cultivation is one of the most important crops and to a lesser extent in the valleys of the rivers Douro and Guadiana. Its incidence varies according to the extension of the breeding places of the vectors and the variations in climatic conditions.

In the regions where the continental type of climate prevails (Douro and Guadiana) there is a moderate anopheline density, the breeding places are confined to pools in the river beds, some swamps, irrigation ditches, etc., and no permanently irrigated crop grows in the regions. The spleen rates are about 15 percent and vivax malaria is the prevailing type of the disease.

spleen rates up to 20 percent and predominance of vivax malaria, Tagus—spleen rates up to 30 percent, vivax and falciparum malaria reaching sometimes up to the same percentage, Sado—spleen rates up to 50 percent with predominance of falciparum malaria in most of the years. Malarial malaria has a very low incidence in all those regions.

## MALARIA VECTORS IN PORTUGAL

Three species of anopheles have been found in Portugal: *maculipennis*, *claviger*, and *nigripes*, the last two being rare and not vectors of malaria in the country.

Of the *maculipennis* complex, two varieties have been found: *typicus* and *atroparvus*.

The *atroparvus* variety is found all over the country. It is a chemical oxygen demand total chlorides per liter sun at least during pools, swamps, drainage and irrigation ditches, and especially in the fields where *atroparvus* breeds in tremendous numbers.

## V MALARIA

ing the woods near the cities. Reforestation was undertaken mainly by planting trees which are not susceptible to the epiphytism of the bromeliads, such as the eucalyptus. This plan has been giving very good results, and in some places permanent results have been obtained. It is, however, a method which cannot be used everywhere and for this reason the National Malaria Service is seeking new methods of combat with the application of insecticides.

(g) During the first 4 months of this year, experimental applications of insecticides were made (DDT and gamexane), as follows:

- I The extra domicile application on the forests and on open places by means of helicopters, of DDT and gamexane in powder and in suspension. These applications showed immediate and good results. Unfortunately, however, we still have no definite conclusions
- II The application of DDT and gamexane in powder and in suspension. These applications showed immediate and good results. Unfortunately, however, we still have no definite conclusions

in most cases were not very satisfactory, especially when the rice plants are high enough to interfere with the distribution of the larvae on the surface of the water.

Better results have been obtained (9, 3) with intermittent irrigation of the fields, but as a good deal of work is necessary for the arrangement of the plots, irrigation and drainage ditches and as in some cases it is necessary to secure a large amount of stored water, the method is not practical in all circumstances within a short time. Sometimes it is even impossible by using it to kill more than 70 percent of the larvae. A mixture of cresol, turpentine, and naphthalene has been also tried (5) with satisfactory results but only in the seedbeds.

With the introduction of the new insecticides DDT and Gamexane, it has become possible to control malaria in the rice field regions by methods employed against either the adult mosquito or its larvae. Various experiments have been made in Portugal by using DDT as well as Gamexane solutions and emulsions as residual sprays in houses, stables, etc., in rural areas with very satisfactory results, as has been done in other parts of the world. However, in spite of the good results obtained, including the effects on houseflies, it is not always easy to spray houses in rural or suburban areas as in butts, barracks, or any kind of animal shelter.

To control malaria in the rice growing regions where house spraying is difficult, or even as an additional measure in mosquito eradication schemes, we attempted to control anopheline breeding in the fields by using DDT as a larvicide. Moreover, as in these regions the fields cover approximately nine tenths of the breeding places, the remaining being formed by irrigation and drainage ditches, it is most cases very easy to know where breeding takes place and where the area to be treated. Even if there are some swampy areas within the region, they are usually not very large and are easily located.

The common larvicide solutions containing DDT were not suitable for application in the rice fields for reasons similar to those related to about paris green. After a number of trials, we found a larvicide with which we could kill anopheline and culicine larvae in the plots by pouring it into the sites where the water enters in and by taking advantage of its diffusibility and differences in tension (surface spreading power is more than 60 times per cent to produce automatic distribution in the fields (7). Further, as water is constantly being drained from one plot into another into the drainage ditches, the currents formed in this way assist in the progress of the larvicide.

Its formula is as follows

1 percent alcoholic solution of DDT - - - - -  
Molhante NNE - - - - -  
Turpentine - - - - -

## V. MALARIA

Breeding starts in spring when the cycle from egg to adult is 25 to 30 days, in summer it is shortened to from 15 to 20 days average of 18 days. Egg and larvae do not appear in any place until the middle of February. Egg laying is suspended in C and the last males die in December.

Systematic larva and pupa counts have shown that the number of larvae is high from April to September, but pupae almost disappear during the second half of June or at the beginning of July, and this time of the year adults only rarely emerge in natural breeding places. The number of larvae increases up to June when an average of over 400 larvae may be found per square meter in the rice fields, the daily production of anophelids at this time of the year is as high as 20,000 adults per hectare. Their effective flight range is at least 1 kilometer, but after 1 kilometer of flight the number of anophelids gradually reduced.

*Atroparvus* feeds on any animal or human being that is accessible though rabbits, pigs, and cattle are its preferred hosts. It feeds only under cover or in its immediate vicinity and never in the open field. For these reasons it only overflows into houses when animals or animal shelters are not sufficient for the number of *anopheles* present in the area.

Sporozoite rates of *atroparvus* caught in houses may reach only 0.12 percent during the summer months, though spleen rates may be almost 50 percent in some rice growing regions where it is the sole vector. This gives an idea of the number of *anopheles* present in those areas.

As Cambournac and Hill have pointed out in 1938, *atroparvus* then is a malaria transmitter in Portugal largely by force of numbers. Any measures which reduce the number of *anopheles* tend to reduce the malaria incidence to an even greater degree.

### METHODS OF MALARIA CONTROL IN THE RICE GROWING REGIONS OF PORTUGAL

Apart from screening of the houses, it is only recently that some methods of malaria control have been found which are of real value in the rice growing regions, due to the difficulty of reducing the larval breeding in the rice plots.

During the late spring and summer months rains are relatively rare in Portugal, and the fields to be planted with rice are plowed early in the spring. The seedbeds are sown in March, the transplantation takes place in May, and harvest is in September. This means that most of the fields are flooded from May to September and that only a small percentage, i.e., the seedbeds, are under water from March to May. If, however, the practice of transplantation is not followed, the fields are kept under water from March to September. In the attempt to control anopheline breeding in the rice fields of Portugal the authors (9) have tried paris green as a larvicide, but the results



This concentrate is diluted 1 to 1 with water before its application, thus forming an emulsion of 1 percent DDT at a cost of 4 escudos per litre

Less expensive yet giving good results is the following

|   | Parts |
|---|-------|
| 8 percent DDT solution in gasoline..... | 25    |
| 10 percent sodium sulphoricate.....     | 10    |
| 10 percent soap solution.....           | 65    |

This is an emulsion of 2 percent DDT that costs 2.80 escudos per litre

Good results have also been obtained with the commercial preparation Larvan containing 10 percent DDT, the price of which is about 15 escudos per litre

Whichever larvicide is used is applied in such a way as to get a final proportion of 1 part of DDT to 20,000,000 parts of water, that is, 5 cubic centimeters of the 1 percent emulsions per 10 square meters (5 litres per hectare).

After trying these mixtures, we came to the conclusion that it was possible to control anopheline larvae in the rice plots by only pouring the larvicide in the irrigation ditches, thus saving much time and labor. The larvae were reduced by 85 percent, and in some cases total elimination was attained, but, due possibly to DDT sedimentation, it was necessary to divide the fields into zones of about 1 to 2 hectares, each one being treated separately.

Another important fact in the treatment of the fields is that the

cide begins to sediment after a period of time, no residual effect of the treatment was observed, the first instar larvae beginning to appear in most cases 5 to 6 days after the treatment. For this reason and as during the summer and treat the plots every 20 o prevent the growth of a second half of July no treatment is necessary for after that time of the year larvae do not grow into pupae, and no adult mosquitoes are then produced.

#### RESULTS OBTAINED IN MALARIA CONTROL

This method has been applied in some areas of several rice growing regions as in Águas de Moura, Pinheiro, Benavente, Alcaçovas, and Vidigueira with similar results.

As time does not allow us to give a detailed account of the results obtained in all these regions, we will only summarize the results ob

After being mixed with water, the larvicide tends to remain superficial layers and is effective against anopheline larvae 1 part in 40 000 000 or 60,000 000 parts of water. But for purposes and as it is not possible to determine exactly the water which is kept in each plot, we have used it in the proportion of 1 part per 20,000 000 parts of water. As the water depth in the fields is about 10 centimeters, 5 cubic centimeters of the larvicide is sufficient to treat an area of about 10 square meters (5 liters per are).

Use of this mixture eliminated the larvae in most of the case from large plots (24 m by 72 m), but it was also seen that the currents were very important in relation to the distribution of the larvicide. Plots wider than 30 meters had to have one inlet for water for each 15 meters where the larvicide was poured and outlets should be located in the opposite sides of those of the inlets (7). Rearranging the irrigation schemes were thus needed. The cost of larvicide was about 12 escudos (50 cents) per liter, and as it was not always possible to get alcohol in the necessary amounts for treating extensive areas experiments with other types of larvicides were undertaken with the intention of making their use more economic and practical.

In view of the fact that the better and cheaper solvents of DDT are not themselves soluble or miscible with water in most cases we decided to give up solutions previously employed and test various emulsions for the purpose.

We also tried DDT solutions in gas oil resin after MacDonald's formula (11), but in some cases the spreading power was too low to avoid damage to the rice plants in the zones of the plots where the larvicide had concentrated.

After a series of trials we found that the most successful results were obtained by emulsifying gasoline or benzene DDT solutions with soap or other emulsifying agents like Puropol and adding substances like Molhante NNF or 10 percent sodium sulphocarbonate to increase the spreading power of the mixture.

The best results were observed with the following formulas

|                                    |       |
|------------------------------------|-------|
| 8 percent DDT solution in gasoline | Parts |
| Molhante NNF                       | 25    |
| 10 percent soap solution           | 10    |
|                                    | 6     |

This mixture is diluted 1 to 1 with water just before its application in the fields, thus forming an emulsion of 1 percent DDT which costs about 2.60 escudos (10 cents) per liter or

|                                    |       |
|------------------------------------|-------|
| 8 percent DDT solution in gasoline | Parts |
| Puropol                            | 100   |
| Water                              | 10    |
|                                    | 200   |

ning of this year if it were possible to treat the fields in the same way as had been done in 1947

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### ABSTRACT OF DISCUSSION

DR HEINRICH MANFRED JETTMAR (Austria) In Chungking when the rice fields were dried up, the larvae of *Anopheles hyrcanus* were segregated in the mud and remained alive up to 36 hours. When they were put into water again, some of them recovered, but they were infected with a *Saprolegnia* like fungus and perished afterwards.

I made the interesting observation that while the larva were stranded on the mud, when the water had gone off the ants streamed  
 Thus the ants destroy the  
 rice field malaria is an  
 cked from all possible

directions

# V. MALARIA

tained in some regions, though malaria was greatly reduced in 1946 where the larvicide has been applied.

In Alcaçovas, for instance, where about 200 malaria cases were recorded in 1946 in the itinerant labourers coming from outside the area to work in the rice fields, only 19 cases have been registered in 1947 when the fields have been treated.

Since 1934 we have made observations concerning malaria in Piheiro, which is an estate with a centrally located town with 212 inhabitants and various houses scattered in the periphery at a distance of about 5 kilometers from the center. In the area between the houses are located about 60 hectares of rice fields. In 1934 the spleen rate was 45 percent and 27.5 percent, respectively. In 1935 the spleen rates were 45 percent and 27.5 percent, respectively. In 1936 the houses of the town were screened, and in 1943 the spleen rate was 26 percent. Since that time the doors have become warped, the screening was damaged, and the spleen rate had risen again in 1945 to 45.4 percent. Weekly *Anopheles* catches in the houses have shown a mean of 3 mosquitoes per house and per visit from June to September of the same year, while in a rabbit pen the mean number of *Anopheles* during the same months ranged between 233 and 278.

In 1946 rice fields began to be partially treated with the larvicides mentioned. *Anopheles* in houses and in the rabbit pen reached only a mean of 0.2 and 20.1, respectively, and the spleen rate was 39.5 percent. The data collected in the dispensary indicated that 12.4 percent of the population of the town and 21 percent of the 25 inhabitants of the periphery had been infected during that year.

In 1947 DDT emulsions were applied from April to July, with an interval of 30 and 20 days between them, to all rice fields within an area of about 3 kilometers around the town (45 hectares approximately), while the remaining 15 hectares in the periphery of the estate were not treated. Each treatment reduced the number of *Anopheles* in the rice fields by at least 65 percent. During that year only 2 *Anopheles* were caught in one house of the town in July, and in the same month their mean number only reached 36.2 in the rabbit pen. The spleen rates made before and after the malaria season were respectively 33.3 and 22.5 percent, and during the year only 3 new cases of malaria were recorded. Thus only 1.9 percent of the population had been found infected. On the other hand, from the 87 inhabitants living in the periphery, 23 percent were infected. *Gambusia* as well as other fishes were present in quantities in the ponds and have not suffered in any way from the larvicide.

In the contrary, *Chironomus* larvae, which are very harmful to young rice plants, were killed in large numbers and could be seen in various parts of the plots treated, forming red patches of 1 sq dm. The same happened to *Dytiscus* and *Hydrophilus*, and people did not complain of their bites in the treated fields. account of this, the rice field owners began to ask at the begin-

press or the public, and the proposal made by the Medical Department in 1945 to eradicate malaria did not receive an immediate response.

However, despite the lack of popular clamour, the Governments of Cyprus and Great Britain considered the scheme worthy of support. Despite the great financial strain on them due to the war, they granted us adequate sums which enabled us to begin this campaign. But it should be noted that the work had to be carried out and expenditure incurred within the framework of the normal financial and administrative procedure.

The task involved the eradication of all anophelines from a moun-

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ro  
gram in two stages, each covering about half the island, to be completed in 1948-49 at a total estimated expenditure of £310,000.

#### ORGANIZATION OF ANOPHELES ERADICATION SERVICE

The director of medical services assigned authority to an executive officer, and by personal observation and by progress reports from the execut . . . . .

Non . . . . . staff consists of headquarters  
staff, larva and imago checkers,  
foremen, and "DDT ers."

*Medical staff.*—There are no medical officers directly attached to the Anopheles Eradication Service (A E S), and no drugs have been issued for suppressing malaria. Persons suffering from this disease obtain their drugs from the various government centers or private doctors. Government medical officers throughout the island render monthly reports of malaria cases examined. Each year an island wide fall survey of spleen and blood parasite rate is made in 140 villages, situated at different altitudes, and with divers types of topography and terrain.

Most of the senior staff of the A E S also carry out a certain

of the principal anophelines were well known. In planning the eradication scheme, the work of Soper and Wilson, concerned with the eradication of *A. gambiae* in Brazil and Egypt, was taken as a guide.



TABLE 3—Comparison of data from Famagusta and Paphos

| District  | 1944            |              |                      | 1945            |              |                      | 1946            |              |                      | 1947            |              |                      |
|-----------|-----------------|--------------|----------------------|-----------------|--------------|----------------------|-----------------|--------------|----------------------|-----------------|--------------|----------------------|
|           | Number examined | Spleen index | Blood parasite index | Number examined | Spleen index | Blood parasite index | Number examined | Spleen index | Blood parasite index | Number examined | Spleen index | Blood parasite index |
| Famagusta | 379             | 70.0         | 38.8                 | 354             | 74.4         | 16.4                 | 679             | 10.7         | 8.8                  | 633             | 6.6          | 0.1                  |
| Paphos    | 900             | 42.2         | 72.3                 | 625             | 33.2         | 26.8                 | 410             | 6.1          | 23.7                 | 1168            | 24.3         | 12.1                 |

1 1946-47 eradication area

2 Control area

Table 4 gives data for fall surveys, 1945 and 1947, of the island

TABLE 4—Species of malaria fall survey

| Number examined | Number positive | Percent | V   | F   | M   | Mixed | Year | Comment  |
|-----------------|-----------------|---------|-----|-----|-----|-------|------|--|
| 1,469           | 1,035           | 21.1    | 630 | 150 | 295 |       | 1945 | Prior to eradication   |
| 4,190           | 215             | 5.0     | 122 | 68  | 25  | 1     | 1947 | Intensive control Nicosia, Famagusta and Ayrenis districts intensive control, Paphos district. |

## Lay-out of Eradication

int  
each  
12 plots

The imago surveyors have to check about 10 percent of the premises in any block, but where they failed to discover any anopheles half, and often all, of the premises are searched by insecticiding. The term "premises" includes all man and animal shelters, natural and artificial caves, pill boxes, tree trunks, etc.

Larva surveyors record the number of units (water surface 5 square yards or less) searched.

Every officer doing field work carries with him a sprayer and insecticide for adult and larvae destruction and also the necessary outfit for collecting them.

## BASIC PRINCIPLES IN ERADICATION

(a) The significance of eradication instead of control

(b) "Positive" finding must be accompanied by a specimen of larvae or imago of any species of anopheles found, with details of the place and date, and the name of the person who actually collects the specimens

(c) Negative—On failing to find anopheles a negative report has to be submitted

## MALARIA

Malaria is not a notifiable disease

Table 1 shows the number of malaria cases reported for the years 1945, 1946, and 1947.

TABLE 1—Malaria cases reported, 1945-47

| Area  | 1945        |               | 1946        |               | 1947        |               |
|---|-------------|---------------|-------------|---------------|-------------|---------------|
|   | Acute cases | Chronic cases | Acute cases | Chronic cases | Acute cases | Chronic cases |
| Karpass <sup>1</sup>                                  | Number 10   | Number 600    | Number 20   | Number 400    | Number 1    | Number 100    |
| Nicosia, Famagusta, Larnaca, and Kyrenia <sup>2</sup> | 301         | 3,361         | 443         | 1,165         | 10          | 491           |
| Rest of the island under intensive control            | 125         | 1,760         | 133         | 2,603         | 28          | 1,295         |

<sup>1</sup> Data for 1945 prior to eradication; for 1946 and 1947 under eradication.

<sup>2</sup> Data for 1945 and 1946, prior to eradication; for 1947, under eradication.

Table 2 shows the spleen and blood parasite rate of school children from three representative villages in the 1946-47 eradication area, Famagusta, and also three from the Paphos District under intensive control.

TABLE 2—Spleen and blood parasite rate of school children

| District               | 1944         |                      | 1945         |                      | Eradication area |                      |              |                      |
|------------------------|--------------|----------------------|--------------|----------------------|------------------|----------------------|--------------|----------------------|
|                        | Spleen index | Blood parasite index | Spleen index | Blood parasite index | 1946             |                      | 1947         |                      |
|                        |              |                      |              |                      | Spleen index     | Blood parasite index | Spleen index | Blood parasite index |
| Famagusta <sup>1</sup> |              |                      |              |                      |                  |                      |              |                      |
| Akanthou               | 30.0         | 30.0                 | 9.3          | 12.9                 | 8.0              | 8.0                  | 6.0          | 0                    |
| Kornokipos --          | 11.0         | 23.7                 | 8.3          | 37.4                 | 0                | 2.0                  | 30.0         | 0                    |
| Ay Andreasikos         | 18.5         | 40.7                 | 8.0          | 10.0                 | 5.0              | 4.0                  | 2.0          | 0                    |
| Paphos <sup>2</sup>    |              |                      |              |                      |                  |                      |              |                      |
| Ay Nicolaos --         | 29.4         | 65.8                 | 20.4         | 15.0                 | 3.1              | 14.8                 | 20.0         | 10.0                 |
| Stravrotopos           | 56.0         | 96.0                 | 56.0         | 35.0                 | 3.1              | 25.1                 | 53.0         | 20.0                 |
| Marathrouda            | 37.5         | 51.5                 | 33.0         | 30.0                 | 2.0              | 18.3                 | 25.3         | 22.4                 |

<sup>1</sup> 1946-47 eradication area.

<sup>2</sup> Under intensive control.

## Blood of infants examined for malaria parasite

1946 eradication area..... 43, all negative.  
 1947 eradication area..... 54, all negative.  
 1947 control area..... 52 examined, 1 positive

While the incidence of malaria in the 1946-47 eradication area, Famagusta, was brought to nil, there has been an appreciable reduction of this disease throughout the island as a result of the intensification of the control measures, as may be noticed from table 3, comparing the two extreme ends of the island.



mated was brought under eradication and protection, (b) increase of wages, (c) extraordinary weather conditions, and (d) delay obtaining larvicides and materials. An additional sum of £25 was therefore granted

### PROBLEMS CONFRONTED FOLLOWING THE COMMENCEMENT OF THE A. E. S.

(a) *Labour*—Increase of wages upsets estimates. Fear of loss of job results in some cases in prolongation of "positive" by deliberate

Shortage of DDT,  
oil, and paris green

necessitating the re-education of the staff in their use, and extra expenditure

(c) *Weather conditions*—Irregular rains create fresh breeding places

(d) *Staff activities*—and re-education

of staff activities

(g) *Black oil and kerosene*—The deterioration of conditions in Palestine, local strikes, and the destruction of the Nicosia power lines affected the work very seriously

(h) *Indirect*—harmful

(i) *Classes*—of

stituted on

anopheles

of anopheles

### COOPERATION

Helpful cooperation is offered by the municipalities and the military authorities. The Conservator of Forests pledged full support



Out of 204 blocks, ranging from 8 to 15 square miles, forming the 1947 eradication area, in about 5 percent a few adult *superpictus* were found at the end of the year in most isolated places. To find these, over 100,000 places were searched by about 200 men.

The following tabulation shows the amount of DDT and other arvicide and insecticide used during the year

| DDT                           | Item   | Amount             |
|-------------------------------|--|--------------------|
|                               | For insecticide and larvicide.....             | 17,000 pounds.     |
| Gas oil                       | Used as larvicide without DDT.....             | 31.5 metric tons   |
|                               | Used as larvicide with DDT 3 to 4 percent..... | 274.0 metric tons. |
|                               | Total gas oil.....                             | 305.5 metric tons  |
| Kerosene                      | .....  | 1,869 gallons      |
| Paris green                   | .....  | 25,536 pounds.     |
| Kerosene, for DDT insecticide | .....  | 932 gallons.       |

*Expenditure on anopheles eradication for the year 1947*

|                  |         |
|------------------|---------|
| Materials.....   | £12,137 |
| Labor.....       | 44,043  |
| Supervisory..... | 10,790  |
| Travelling.....  | 8,014   |
| Total.....       | 73,920  |

By the end of 1947 in most months the number of 52 blocks were during 1946-47  
Strict check

found positive  
for all species of anopheles, week by week, within the 1946-47 and 48 eradication areas.

TABLE 5—Blocks examined 1946-47 and 1948 eradication areas

| Date | Number of blocks    |     |     |                  |     |     |
|------|---------------------|-----|-----|------------------|-----|-----|
|      | 1946-47 eradication |     |     | 1948 eradication |     |     |
|      | Neg                 | Pos | Uns | Neg              | Pos | Uns |
| 10   | 160                 | 2   | 42  | 125              | 72  | 157 |
| 17   | 151                 | 1   | 52  | 115              | 85  | 149 |
| 24   | 179                 | 1   | 24  | 110              | 90  | 143 |
| 31   | 179                 | NB  | 24  | 149              | 119 | 84  |
| 7    | 169                 | NB  | 35  | 163              | 109 | 80  |
| 14   | 169                 | 1   | 34  | 174              | 83  | 95  |
| 21   | 167                 | NB  | 37  | 195              | 64  | 73  |
| 28   | 174                 | 1   | 29  | 221              | 64  | 47  |
| 6..  | 184                 | NB  | 20  | 218              | 93  | 41  |

ent checkers and guide the A E S staff to locate isolated breeding places

The species of anopheles mosquitoes found in the 1917 eradication area are *A. superpictus*, *A. elutus*, *A. bifurcatus*, *A. algerienses*, *A. marteri*, *A. multicolor*

### STAFF

The staff employed in the 1917 A E S consisted of—executive officer, 1, clerks, 6, storekeeper, 1, malaria technicians 2, headquarters inspectors, 3, pay officer, 1, district officers, 3, field inspectors, 4 section officers 15, zone officers, 15, regular laborers (temporary zone officers, larva and adult surveyors), 132, casual laborers, 233 (monthly average)

### START OF ERADICATION

During the 1916-17 winter months every effort was made to destroy *A. superpictus* and *A. elutus* adults by light treatment of all possible sheltering places with gas oil containing 3 to 4 percent DDT, by Hudson and small hand sprayers. The principal places sprayed were stables, sheepfold, pigsties, caves, gardeners' sheds, and, where possible, some sleeping rooms. In some of the most malarious villages, efforts were made to spray all houses. This work was preceded by checking and recording the presence of anopheles. A few villages were sprayed with plain gas oil, and a number of premises were left altogether unsprayed as a control but on finding anopheles in these facts they were all sprayed with DDT before the breeding season

Following spraying with DDT, adult checkers with hand insecticide sprayers commenced searching for adult anopheles, using a sheet to collect knock down mosquitoes. Checking for the presence of anopheles larvae continued even during the height of the winter months, with the object of detecting the first generation of *A. superpictus* and *A. elutus* larvae. General treatment of all breeding places commenced thereafter with gas oil containing 3 to 4 percent DDT on a 21 days' cycle, according to altitude. Any drains existing in such a marshy areas were treated with larvicide by ordinary sprayers, so drainage work was carried out. Any drains existing in such areas were cleared in order to facilitate larviciding. Spectra of early eradication were good, but shortage of essential funds seriously curtailed operations during the most critical time of February. It is noticed that some adults could be found in most unexpected breeding season such as holes in the outer walls of buildings, cracks of rocks, and old uninhabited caves, and trunks of trees near and far. Some of these sheltering places, which were not sprayed. Some of these sheltering places could be sprayed only by suspending a man with ropes

just within the last few weeks returned from a critical visit to the island. As it is not only of extreme importance to Cyprus as an example to other places as well, I think we should try to overcome some of the difficulties encountered.

One of the first difficulties, as Mr Aziz said, was that the Government, there was no state of national emergency which would justify the measure and which made possible any form of dictatorial law giving excessive powers to government. The work has all been done strictly under the normal powers conferred. Secondly, and for the same reasons, this is very strictly a local effort made by the people of Cyprus without any special importation of staff. They had to increase their own staff mainly in the lower ranks, but in the senior and medium executive staff they have relied on the personnel of the public health department.

Thirdly, it has had to be done at extremely low costs. From Mr Aziz that it now averages something more than \$1 per person protected and is not likely to rise to more than \$2.50 per head when the time it is all completed.

the anopheles makes the discovery and treatment of the disease extremely difficult.

One of the interesting things that came out of this work was the question of the prevention of reimportation, and I think this must be adequately tackled. It will obviously require the destruction of the mosquito wherever it is found, whence the disease is brought in, to make it impossible for the disease to spread to other parts of the island.

considered. Instead of having all nations developing independently, it is better to have a few large conventions.

interested in the paper by Mr Aziz and really come to the point where I can take just 1 minute of time to pay tribute to him. I am sure that Mr Aziz is a man of great ability and I am sure that he will be a great help to the world.

of Cyprus

Further, you might be interested in the fact that the work which Mr Aziz, because of his modesty, has neglected to mention, Mr Aziz, I have been informed was an assistant of Sir Ronald Ross.

the 1947 eradication area was For *A. superpictus* larva, October 1947, adult, January 21, 1948 For *A. elutus* larva, July 14, 1948 adult, June 2, 1947

# SUMMARY

The possibility of eradicating malaria from an area of approximately 2,000 square miles with an approximate population of 300,000 at an expenditure of £75,000, or 5s per head, without any drainage issuing of any suppressive drugs, by attacking adult and larvae of several species of indigenous anopheles vectors was demonstrated. Although DDT is a powerful contact insecticide and valuable as larvicide, the success of eradication depends largely on careful planning and checking. Knowledge of the habits of the respective species of anopheles, as well as of local conditions, is important. Much depends on the facilities in obtaining adequate essential supplies such as DDT, oil and sprayers.

The decline of incidence of malaria even from highly malarious villages has been very rapid.

The cooperation of the inhabitants is limited to allowing their premises and water supplies to be sprayed. The eradication of malaria and domestic pests such as fleas, flies, bugs, and mosquitoes by regular insecticiding is a relief to them and their animals, and is highly appreciated.

*A. elutus* the marsh breeder, has not been found throughout the island during the last 6 months.

The occasional finding of a few adult *A. superpictus* in certain blocks, formerly negative over a considerable period, is suspected to be due to the wind and the free movement of animals, vehicles, and coastal craft in and out of the eradication area.

The 1946-47 eradication areas are being frequently and strictly checked by a group of experienced men, while anopheles in the 1948 area is being eradicated.

The cooperation of the municipalities and particularly of the Forest Department staff and the military authorities is much appreciated. The difficulty confronted in mountainous areas in discovering breeding places and the adaptability of certain species of anopheles to the natural and artificial sheltering places away from the habitations of the people are great. The habit of the people to live in the away from their homes and often far from their villages is a problem if one has to rely on the spraying with DDT of the interior of village houses.

## ABSTRACT OF DISCUSSION

GEORGE MACDONALD (United Kingdom) Through the kindness of the director of medical services of Cyprus I have been kept in close contact with this work since it started and have only

### Session 3 CHEMOTHERAPY

Friday, May 14—9 30 a m. to 12 m.  
Departmental Auditorium, Main Hall

The chairman, Sir Gordon Covell, announced that an exhibit of specimens of malaria parasites, prepared by Mr C P Shute, was being set up in the foyer. Mr Shute was unable to be present but sent the material from England.

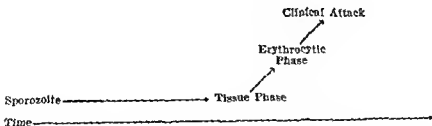
### SCREENING TESTS AND THE PHARMACOLOGIC APPRAISAL OF PROSPECTIVE PLASMOCIDES

JAMES A SHANNON, *The Squibb Institute for Medical Research,  
New Brunswick, N J*

It is possible, as the result of work during the past 8 years, to approach the development of more effective antimalarials in a more rational manner than heretofore. This is largely because of a better appreciation of the biology of the various malarial parasites, their responses to chemotherapeutic agents, and the natural history of the

co 1—The natural history of malarial infection and toxicity  
(c) in malaria

Chart 1—*Falciparum malaria*



- (a) Prophylactic, which is manifest against the sporozoites or the parasites of the primary tissue phase;
- (b) Suppressive, which is manifest against the asexual parasites of the erythrocytic phase, and
- (c) Gametocidal, which is manifest against the gametocytes of the erythrocytic phase

In vivax malaria (chart 2), because there is a persistence of the tissue phase of the disease, a fourth type is possible. This is commonly called curative antimalarial activity and is manifest against

## V MALARIA

in 1913 when the latter made his survey of that island. That say, Mr Aziz has been at this job for at least 34 years. When Barber and Dr Rice worked in Cyprus in 1935, Mr Aziz was their right hand man. Later for Mr Carter and myself, he also the one who did the work.

For myself, I am very much interested in the progress that has been made in anopheles reduction and apparent reduction in malaria incidence, but I seems to me we can still maintain some scepticism and will still be interested in future results, that is, to know how much of this decrease may be attributable to natural changes in malaria incidence, but anopheles and malaria are eradicated from Cyprus, I will certainly take off my hat to Mr Aziz, his coworkers, and to the Government of Cyprus.



malaria, a detectable prophylactic activity in vivax malaria, a high order of suppressive activity in both infections, but no curative activity in vivax malaria, and no demonstrable gametocidal activity in falciparum malaria. It does have an effect upon the falciparum

assistant. This does not appear to be the case with the other drugs so far discussed (2, 3).

The investigations upon which this summary of activities is based have no doubt that these various antimalarial activities differ one from the other in a wholly qualitative fashion. That is to say, the possession by a drug of

and one to expect high

that the biological me

with the suppressive activity of quinacrine and chloroquine are related

manifest in one infection is of equal importance to the maintenance of the life of a parasite of another infection.

These facts make the study and development of new plasmocidal

antimalarial activities in the human host. These testing procedures have been utilized in the examination of a fairly large number of speculative antimalarials during the past 6 years so that a reasonable definition of the predictive value of the common screens has been achieved.

Time does not permit a detailed review of the many host-parasite relationships which are available for the preliminary examination of speculative antimalarials. This has been ably done in Dr. Wiselogle's monograph. Generally speaking, the data contained therein indicate

that, in the preliminary examination of antimalarials, only a single human screen is

the discovery

covery of pro

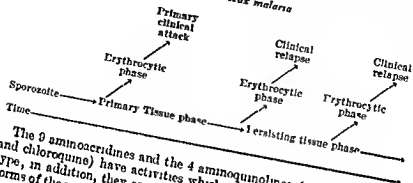
is lack of cor

relation of prophylactic and curative activity as observed in the

<sup>1</sup> *Cynomolgus* malaria in the rhesus monkey may be an exception. However the variation in response of the many host-parasite relationships to drugs including vivax and falciparum malaria makes this an unlikely possibility. This subject is now under careful study by Dr. Leon Schmidt, Christ Hospital, Cincinnati.

the persisting tissue parasites, such as those which appear to be responsible for the periodic release of new lines of parasites which invade the blood and initiate relapses as discrete episodes in the course of the infection

Chart 2.—*Vivax malaria*



The 9 aminoacridines and the 4 aminoquinolines (e.g., quinacrine and chloroquine) have activities which are primarily suppressive in type, in addition, they exert a plasmodicidal effect against the sexual forms of the vivax infection (table 1). They have no activity against the parasites of the primary tissue phases of vivax and falciparum or against those of the persisting tissue phase of vivax. Hence, they have no prophylactic or curative activity. The 8 aminoquinolines, (e.g., pamaquine, pentaquine, and isopentaquine), exert an extraordinary degree of activity against the gametocytes of the falciparum infection, a usable degree of curative activity in vivax malaria, and are both infections. From the standpoint of practical utility, then, it could appear that the 9 aminoacridines and the 4 aminoquinolines are one group of activities, and the 8 aminoquinolines another. The separation of these activities is fortuitous and not indicative of qualitative grouping of susceptible biological systems within the parasites. This is clear from a consideration of chloroguanide. This drug has a very high order of prophylactic activity in falciparum

TABLE 1.—Practical antimalarial activities of representative drugs<sup>1</sup>

| Malaria       | 8-amino-quinolines | 4-amino-quinolines | Chloroguanide |
|---------------|--------------------|--------------------|---------------|
| Prophylactic  | +                  | +                  | +             |
| Suppressive   | +                  | +                  | +             |
| Plasmodicidal | +                  | +                  | +             |
| Curative      | +                  | +                  | +             |

<sup>1</sup> It is made to compare relative activities except in the case of prophylactic activities of chloroguanide. Here, although prophylactic action is not complete, it would appear to be supporting the suppressive activity of the drug in routine field suppression.

that the partial correlation of the activity of chlorguanide in gallinaceum in the chick and in the human infections falls down completely in relation to pamaquine, pamaquine has no detectable prophylactic activity in the gallinaceum infection. The purely suppressive drugs, quinine and quinacrine, have no detectable prophylactic activity in either the avian or human infections.

In résumé, then, it would appear to be possible to have high prophylactic activity in the avian infections and no prophylactic activity in the human infections, or a high prophylactic activity in the avian infections and in the human infections, or, finally, little or no prophylactic activity in the avian infections and moderate prophylactic activity in the human infections.

Various screening devices have been used to study the curative activity of drugs in malaria. The most commonly used is the standardized test in the canary.

by Drs Robert Courtney and Joseph Greenberg in terms of the minimal effective doses of sporozoites or erythrocytic parasites which will produce an infection curable by the maximally tolerated dose of a drug (5). Using such an avian screen with some of the current drugs, one obtains varying degrees of curative activity, as summarized in table 3. It is im-

curative activity

difference between

guanide as measured in this screen, pamaquine has usable curative activity in vivax malaria, whereas chlorguanide has none. A similar lack of correlation can be shown by the use of other data derived from other avian screens. Actually, no drug studied during the war years which was specifically selected because of a curative activity in one or another avian screen, was shown, by direct examination in the man, to possess a comparable activity in the human vivax infection. It would appear that some correlation may emerge, at least within an homologous series, from the study of the curative activity of drugs

TABLE 3.—Differences in drug response, curative activity\*

| Drug        | Parasite and host   |             |
|-------------|---------------------|-------------|
|             | Gallinaceum (chick) | Vivax (man) |
| D. Malarine | +                   | 0           |
|             | +++                 | 0           |
|             | +++                 | 0           |
|             | +++                 | +++         |
|             | ++                  | 0           |

\* The relative activity of drugs in the gallinaceum infection in the chick are from the experiment of Courtney and Greenberg (5). The relative activity of drugs in the human infection are from the experiment of Courtney and Greenberg (5).

avian infections, and those observed in human vivax and falciparum malaria.

There is summarized in table 2 a series of representative compounds derived from seven different chemical series. These compounds were studied for prophylactic activity in several species of experimental malaria, and some in falciparum malaria in human beings. In three different

There is summarized in table 2 a series of representative compounds derived from seven different chemical series. These compounds were studied for prophylactic activity in several avian screens, in vivax malaria, and some in falciparum malaria. The avian screens included three different parasites and three different hosts. The first four compounds all showed a very high order of prophylactic activity in the gallinaceum infection in the chick. These compounds were also found to have some degree of activity in the cathemerium infection in the turkey. However, the first canary and in the lophurae infection in the turkey. However, the first three compounds on the list showed no prophylactic activity in vivax malaria. The fourth compound, chlorguanide, had a similar high

TABLE 2.—Differences in drug response prophylactic activity:

| Drug                 | Parasite and host          |                           |                            |                          |                  |
|----------------------|----------------------------|---------------------------|----------------------------|--------------------------|------------------|
|                      | Cathem-<br>rum<br>(canary) | Gall<br>narium<br>(chick) | Lepthar-<br>um<br>(turkey) | Falcip-<br>arum<br>(man) | V. luei<br>(man) |
| Butadiamine          | +                          | +                         | +                          | 0                        | 0                |
| 5-Chloro-2,4-diamine | +                          | +                         | +                          | +                        | +                |
| Metachloridine       | +                          | +                         | +                          | +                        | +                |
| Chloroguanide        | +                          | +                         | +                          | +                        | +                |
| Amprolium            | +                          | +                         | +                          | +                        | +                |
| Amprolium            | +                          | +                         | +                          | +                        | +                |
| Quinine              | +                          | +                         | +                          | +                        | +                |
| Ulnacrine            | +                          | +                         | +                          | +                        | +                |

Prophylactic activity is expressed as 0, +, or ++. 0 indicates the lack of a detectable prophylactic activity; + in brackets the presence of an action which is incomplete.

|  |   |   |   |     |    |
|--|---|---|---|-----|----|
|  | + | 0 | + | +++ | 0  |
|  | 0 | 0 | 0 | ++  | ++ |
|  | 0 | 0 | 0 | +   | +  |
|  | 0 | 0 | 0 | 0   | 0  |

Prophylactic activity is expressed as 0 + or +++ 0 indicates the lack of a detectable degree of prophylactic activity + indicates the presence of an action which is incomplete +++ indicates possession of complete prophylactic action

prophylactic activity in gallinaceum infection  
 activity in the cathemerium infection  
 in falciparum

phylactic activity in gallinaceum infections, a barely detectable activity in the catheumerium infection, and a very high order of activity in falciparum malaria. However, a rather striking difference was noted in the prophylactic activity of this compound in falciparum vivax malaria. In the latter infection, the maximally tolerated dose of chloroguanide (1 c, 10 gram), administered for 7 days, was able only to produce a short delay in the appearance of erythrocytopenia, whereas, in falciparum malaria, a single dose as small as 100 milligrams, administered on the second to the fifth day of the infection, produced complete prophylaxis (4).

The remaining compounds in table 2 are also of interest. Chloroquine, a detectable degree of prophylactic activity in the lophurae infection in the turkey, this is slight, but, at the same time, is no greater than the prophylactic activity which can be demonstrated for pamaquine in catheumerium malaria in the canary and in lophurae malaria in the turkey. Nonetheless, chloroquine has no prophylactic activity in falciparum or vivax malaria, whereas, pamaquine, at high doses, is a complete prophylactic action in each infection. It is particularly important to note, in connection with the latter drug,

Table 5 summarizes the comparative suppressive antimalarial activities of these compounds in *gallinaceum malaria* in the chick, *lophuræ malaria* in the duck, and *vivax malaria* in man. All active

those of SN 7618 in *lophuræ malaria* in the duck and higher than 7618

TABLE 5—Differences in drug response suppressive activity of substituted 4 aminoquinolines

| Survey No. | Chloroquine equivalents |                |             |
|------------|-------------------------|----------------|-------------|
|            | Gallinaceum (chick)     | Lophuræ (duck) | Vivax (man) |
|            | 0.15                    | 0.15           | 0.15        |
|            | 2                       | 2              | 2           |
|            | 15                      | 4              | 3           |
|            | 10                      | 10             | 1.0         |
|            | 1.00                    | 1.00           | 1.00        |
|            | 1.5                     | 2              | 2           |
|            | 1.5                     | 4              | 2           |
|            | 5-2.0                   | 10             | 6           |
|            | 1.5                     | 10             | 10          |
|            | 1.0                     | 2              | 1.0         |
|            | 1.0-3.0                 | 2              | 1.0         |

In the 1 man vivax infection 7618. Had the the selection of two compounds n error, since it the other hand, the basis for the selection of compounds for trial in man, these two compounds stand out as the two better compounds of the series. Similar data could be presented for other chemical series wherein the prediction value of the gallinaceum infection was less than that of some other host-parasite relationship. As far as one is able to determine from the data, there is no single best avian screen. It is essential even in the study of compounds within a homologous series to utilize more than a single avian screen in the selection of compounds for trial in man. It is also essential to study in man a number of the better compounds, as judged to

the  
but



SN 7618 has been taken as the reference standard. Two animal toxicity tests were used, one a 7 day mouse test, the other, an 11 day rat test. Both may be considered to measure short term chronic toxicity. In the human experiments, drug was administered twice daily over a number of weeks, with progressive increments in dosage until the maximal dosage generally tolerated was determined.

TABLE 6.—Differences in drug response toxicity of substituted 4-aminoquinolines

| Survey No. | Mouse 7-day test (chloroquine equiv. grams) | Rat 11-day test (chloroquine equiv. grams) | Man tolerated daily dose |
|------------|---|--|--------------------------|
|            | 0.6   | 0.3  | Grams > 0.8              |
|            | 1.0   | 1.0  | 4                        |
|            | 1.0   | 1.6  | 3                        |
|            | 1.00  | 1.00                                       | 4                        |
|            | 5   | 5  | > 4                      |
|            | 1.0   | 1.0  | 3                        |
|            | 3   | 6  | 3                        |

Drugs SN 3294 and SN 8137 are characterized by a lower toxicity in the mouse and in the rat than SN 7618, and it is apparent in man that they also have a lower toxicity, since the maximally tolerated dose in each case is better than 50 percent in excess of the maximally tolerated dose of SN 7618. However, if one takes SN 7135 and SN 9584, the situation is reversed, that is to say, the toxicities in the mouse and in the rat are no greater and may be less than SN 7618. However, the maximally tolerated dose for man is significantly less. These variations, by and large, are within a factor of 2 and can be uncovered only by fairly carefully controlled observations in man. However, the importance of such observations lies in the fact that a factor of 2 in terms of toxicity may be the deciding factor in the determination of which of several drugs under study is the best. Apart from this consideration, the mouse and rat toxicities have, in general, fair prediction value for the situation obtaining in man.

This is also true for a number of the 8 aminoquinolines which were studied. The structures of a representative series are listed below (table 7).

These were studied for toxicity in the rat, some were also studied for toxicity in the mouse, and all were studied for tolerability in man. The data on the toxicity of these compounds (table 8) have been calculated using SN-971, or pamaquine, as the reference standard. A consideration of these data leaves no doubt that general toxicity, as shown by the mouse and rat, has reasonable prediction value for man. There are exceptions in this table, but few exceed a factor of about 2, the greatest difference being in the case of the last compound, SN-13694, which on the basis of the data available, would appear to exceed a factor of 6.

## V MALARIA

study of antimalarials during the war that it was necessary to utilize quantitative techniques which would permit the study of the physiological disposition of a speculative agent early in the study of a compound or series of compounds. Such study is essential in the determination of whether a compound is absorbed or not absorbed, the extent to which it is localized in the tissues of the body, and rates at which it is degraded and excreted. Generally speaking, drugs which are degraded or excreted very rapidly require more frequent dosage than those which are localized extensively in the tissues and which are degraded and excreted at a very low rate. On the other hand, drugs falling in the latter category require large initial loading doses as compared with drugs falling in the former category. Such information can be obtained only in the human subject to be applicable to the study of the effectiveness of a speculative agent in human malaria. However, it is possible to obtain valuable information from carefully controlled animal work particularly if performed on the larger animals, such as the dog or the monkey.

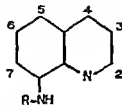
This aspect of pharmacological work up of a speculative agent is to be emphasized, since in the absence of such information it is frequently difficult to obtain clear-cut information on the presence or absence of prophylactic activity and on the degree of suppressive activity in simple experimental routines. Such information is as important in designing a dosage regimen in the preliminary examination for antimalarial activity as it is at a later time in designing the routine dosage schedules which will be used in practice in the field.

The toxicity of an antimalarial is obviously as important in determining its general utility as its antimalarial activity. Consequently, a brief time will be spent in considering the usefulness of information which can be derived from the usual laboratory examination of this type. It is unusual for one to be able to translate toxicity data obtained in the small laboratory animals directly to the situation which will obtain in man. This is, in part, because the phenomena which are attendant on the administration of high dosages in experimental animals are not usually the phenomena which impose an upper limit on drug dosage in man. However, the importance of doing so is also appreciated that, at a later date, specific toxicity experiments must be performed in man if one is to determine the relationship between the dosage which will be generally effective and the dosage which will be generally tolerated by the human subject. This is supported by the data obtained in the study of a number of quinolines previously discussed which were carefully examined for toxicity in the human subject (table 6). Again, as in the case of suppressive antimalarial activities of these compounds,





TABLE 7—Structures of a series of substituted 8-aminoquinolines studied for toxicity in several hosts

|  |                     |                     |   |
|---|---------------------|---------------------|---|
| Survey No.  | R                   | S                   | N   |
| 871   |                     | CH <sub>3</sub> -O- | (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> N-(CH <sub>2</sub> ) <sub>2</sub> -CH(CH <sub>3</sub> )-                |
| 1 452   |                     | CH <sub>3</sub> -O- | H <sub>2</sub> N-(CH <sub>2</sub> ) <sub>2</sub> -  |
| 8 233   | CH <sub>3</sub> -O- | CH <sub>3</sub> -O- | (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> N-(CH <sub>2</sub> ) <sub>2</sub> -CH(CH <sub>3</sub> )-                |
| 9 972   | CH <sub>3</sub> -O- | CH <sub>3</sub> -O- | (CH <sub>3</sub> ) <sub>2</sub> CH-NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(CH <sub>3</sub> )-                          |
| 11 191  |                     | CH <sub>3</sub> -O- | (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> N-(CH <sub>2</sub> ) <sub>2</sub> -                                     |
| 11 226  |                     | CH <sub>3</sub> -O- | (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> N-(CH <sub>2</sub> ) <sub>2</sub> -NH-(CH <sub>2</sub> ) <sub>2</sub> - |
| 12 352  |                     | CH <sub>3</sub> -O- | H <sub>2</sub> N-(CH <sub>2</sub> ) <sub>2</sub> -  |
| 12 354  | CH <sub>3</sub> -O- | CH <sub>3</sub> -O- | H <sub>2</sub> N-(CH <sub>2</sub> ) <sub>2</sub> -  |
| 12 451  |                     | CH <sub>3</sub> -O- | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -                     |
| 13 232  |                     | CH <sub>3</sub> -O- | (CH <sub>3</sub> ) <sub>2</sub> CH-NH-CH <sub>2</sub> -   |
| 13 233  |                     | CH <sub>3</sub> -O- | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -                     |
| 13 274  |                     | CH <sub>3</sub> -O- | (CH <sub>3</sub> ) <sub>2</sub> CH-NH-(CH <sub>2</sub> ) <sub>2</sub> -CH(CH <sub>3</sub> )-                          |
| 13 276  |                     | CH <sub>3</sub> -O- | (CH <sub>3</sub> ) <sub>2</sub> CH-NH-(CH <sub>2</sub> ) <sub>2</sub> -   |
| 13 380  |                     | CH <sub>3</sub> -O- | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -                     |
| 13 429  |                     | CH <sub>3</sub> -O- | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -                     |
| 13 694  | Cl                  | CH <sub>3</sub> -O- | (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> N-(CH <sub>2</sub> ) <sub>2</sub> -                                     |

These data emphasize the rough prediction value of careful toxicity studies in the routine work up of potential agents in the small laboratory animals, particularly in the comparative study of compounds within an homologous series. However, again in the selection of the best compound within a series, such data cannot be substituted for the direct study of tolerability in man.

One other point relative to toxicity studies is of importance. Data such as are shown in table 8 give little information of the qualitative nature of the toxicity which is to be expected in man. An indication of this can usually be obtained from the study of the toxicity of drugs

TABLE 8—Differences in drug response toxicity of substituted 8-aminoquinolines

| Survey number          | Pamaquine equivalents |                 |                            |
|------------------------|-----------------------|-----------------|----------------------------|
|                        | Mouse 7-day test      | Rat 14-day test | Man, 14-day administration |
| 871                    | 1.00                  | 1.00            | 1.00                       |
| 1 452                  | 5                     | 3               | 4                          |
| 8 233                  | 1.0                   | 5               | >1.0                       |
| 9 972                  |                       | 5               | 2                          |
| 11 191                 | 5                     | 5               | <1.0                       |
| 11 226                 |                       | 5               | 2                          |
| 12 352                 |                       | 4               | 2                          |
| 12 354                 | 5                     | 5               | 2                          |
| 12 451                 |                       | 6               | 5                          |
| 13 232                 | 5                     | 5               | 5                          |
| 13 233                 |                       | 5               | 5                          |
| 13 274 (isopentaquine) |                       | 5               | 5                          |
| 13 276 (pentaquine)    | 6                     | 5               | 5                          |
| 13 380                 |                       | 3               | 5                          |
| 13 429                 |                       | 3               | 5                          |
| 13 694                 | --                    | 6               | <1                         |

in the larger animals. Actually, it was from such studies on dogs and monkeys in the wartime malaria program that plasmocide and certain related compounds were excluded as potential leads in the exploration of the utility of the 8 aminoquinolines as curative agents in vivax malaria. The data which led to the discard of this type compound were the clear cut demonstration that plasmocide and certain analogous compounds produce discrete irreversible lesions in the central

compounds

It would be very fine were one able to look back on the efforts and accomplishments in the field of the chemotherapy of malaria during the past 10 years and evolve, from the vast amount of information which has been collected, a simple, dependable, and direct routine with which to study new speculative antimalarial agents. This does not appear to be the case. On the other hand, there is a reasonable, though still empirical, basis for further study.

Such an approach is time-consuming, costly, and not without its

problem at the present time

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- (4)
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# CHLOROQUINE<sup>1</sup>

DAVID P. EARLE, JR. and ROBERT W. BERLINER, *Department of Medicine, New York University College of Medicine*

## INTRODUCTION

Certain derivatives of 4-aminoquinoline were among the more promising of many synthetic compounds studied during the wartime malaria research program. The 4-aminoquinolines had been considered as potentially useful antimalarial agents in several countries prior to the war (1, 2, 3), but the drugs had not received adequate pharmacological or clinical study.

During the course of malaria studies in this country, Blanchard expressed interest in the potentialities of these compounds (4), based on a consideration of the chemical structure of quinacrine and related compounds. He believed that derivatives of simpler nuclei should possess antimalarial activity and viewed the relationship of two such chemical series to quinacrine as shown in figure 1. However, it was not until the French in North Africa found one of the 4-aminoquinolines (santoquin, SN-6911) to have good activity in human malaria

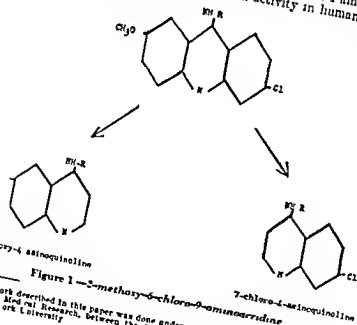


Figure 1—2-methoxy-6-chloro-9-aminoquinoline

Work described in this paper was done under a contract recommended by the Committee on Malaria Research, between the Office of Scientific Research and Development and New York University.

at well tolerated doses, that any serious effort was made in this country to explore the series

Following this stimulus, a large number of derivatives of 4 amino quinoline was synthesized and examined for antimalarial activity in avian malarials. The 10 compounds selected for trial in man are shown in table 1. It is the purpose of this communication to discuss the considerations that led to the selection of chloroquine (SN-7018) as the most useful of the group and then to present in more detail the pharmacology, toxicity, and clinical use of this effective antimalarial agent

TABLE 1—Structure of the substituted 4-aminoquinolines studied

| Name      | Survey number | Nuclear substituents | Substituent on 4-amino group |
|-----------|---------------|----------------------|------------------------------|
| Santoquin | RN-3294       | 6-methoxy            | d-ethylamino-1-methyl-butyl  |

### PROCEDURES

The assays of antimalarial activities were carried out against blood induced infections of *P. vivax* (McCoy strain) and *P. falciparum* (McClendon strain) malaria in susceptible individuals. The response of these strains of malaria to quinine and quinacrine treatment is known. The testing procedures have been demonstrated to yield reproducible results (5, 6). The activity of related compounds may be compared to one another as well as to quinine and quinacrine by these procedures. Antimalarial activity assayed in this manner is probably a true measure of the ability of an agent to suppress and cure naturally occurring falciparum malaria and to suppress naturally occurring vivax malaria. The absolute values for activity, however, are not directly applicable to all strains of plasmodia.

The therapeutic tests were performed in accordance with standard procedures previously outlined (5, 6). The regimens of dosage were designed to produce fairly stable plasma drug concentrations during the 4 day (vivax) or 6 day (falciparum) therapeutic period. All doses in this paper are given in terms of the free base. Therapeutic results were classified in three groups: class 1, no certain effect, class 2, temporary suppression of parasitemia and/or fever, class 3, permanent effect, i. e., absence of parasitemia for 14 days (vivax) or 21 days (falciparum), followed by a positive reinoculation to indicate continued host susceptibility to the infection.

# CHLOROQUINE<sup>1</sup>

DAVID P. EARLE, JR. and ROBERT W. BERLINER, *Department of Medicine, New York University College of Medicine*

## INTRODUCTION

Certain derivatives  
using  
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It is well known that derivatives of simpler nuclei possess antimalarial activity and related compounds have been synthesized and tested.

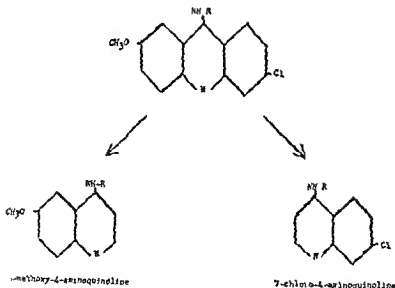


Figure 1 — 2-methoxy-6-chloro-9-aminoacridine

The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and New York University.



## SELECTION OF CHLOROQUINE

The relative antimalarial activity of the seven 4 aminoquinolines studied in our laboratories for action against McCoy strain vivax malaria is shown in table 2. Activity is recorded in terms of both the lowest total oral dosage and lowest mean plasma drug levels required to achieve class 3 effects, i. e., permanent eradication of parasitemia. In addition, santochin, chloroquine, oxychloroquine, and SN-10751 were assayed against McClendon strain falciparum malaria. Their antimalarial activity was of the same order as had been found in vivax malaria, but larger total doses and higher plasma drug levels were required for permanent eradication of parasitemia.

TABLE 2—Antimalarial activity of certain derivatives of 4 aminoquinoline in blood induced vivax malaria (McCoy strain)

| Drug           | Number of patients studied | Required to eradicate erythrocytic phase |                               |
|----------------|----------------------------|--|-------------------------------|
|                |                            | Lowest total dose                        | Lowest mean plasma drug level |
|                |                            | Grams                                    | Micrograms per liter          |
| SN-3294        | 8                          | 2.1                                      | 350                           |
| SN-7135        | 6                          | 1.1                                      | 120                           |
| Santochin      | 19                         | 1.1                                      | 50                            |
| SN-9584        | 13                         | 7  | 15                            |
| Oxychloroquine | 15                         | 375                                      | 17                            |
| SN-10751       | 14                         | 35                                       | 19                            |
| Chloroquine    | 25                         | 30                                       | 10                            |
|                |                            | 275                                      |                               |

The drugs are listed in order of increasing effectiveness.

The differences between the activities, on the basis of total oral doses, of the four most active compounds are not great. Accordingly, chloroquine, oxychloroquine, SN-9584, and SN-13425 were subjected to a comparative assay for toxicity in normal young adult volunteers. Chloroquine was administered to 32 men while each of the other drugs was given to a group of 16 men. Rations of 50 milligrams were given in equal doses each day during the first week. 100 milligrams the second week, and an additional 100 milligrams each subsequent week. A daily increasing booster dose was given on the first day of each week. The drugs studied are listed in table 3, in order of increasing toxicity.

TABLE 3—Toxicity of certain derivatives of 4-aminoquinoline

| Drug        | Highest daily oral dose test | Number of subjects   | Chief character of symptoms   |
|-------------|------------------------------|----------------------|---|
| Chloroquine | Grams<br>0.6<br>4<br>4<br>4  | 16<br>16<br>16<br>16 | None<br>Difficulty in visual accommodation<br>Pruritus, nervousness and anxiety<br>Pruritus, nervousness and anxiety, nausea and vomiting |

The drugs are listed in order of increasing toxicity.



site free intervals were 15 days for santochin and oxychloroquine, and 36 days for chloroquine. The plasma drug concentrations of all 3 drugs at the end of the prepatent periods were below the minimal effective suppressive level of the drug studied. It appears likely, then, that a weekly dose of 0.25 grams of any one of the three drugs is close

quine in falciparum malaria with similar results

### SPECIFIC STUDIES WITH CHLOROQUINE

*Pharmacology*—The physiological disposition of chloroquine was

Absorption from the gastrointestinal tract and excretion by the kidneys were examined in balance studies in which the subjects received the drug over a period of days until the plasma drug concentrations had become stable. Urine and stool collections were made during the last 48 hours of drug administration. An average of only 8 percent of the daily dose was recovered from the stools, indicating fairly complete absorption. Urinary excretion under ordinary conditions accounted for 10 to 25 percent of the daily oral dose. However, the rate of renal excretion was varied over a wide range by the

the plasma chloroquine concentration falls following the termination of therapy. This decline amounts to approximately 50 percent every week.

The distribution of chloroquine was examined in the body fluids and tissues of young adult albino rats and dogs. Plasma and tissue samples were obtained 24 hours after the last dose of a series of oral administrations over a period of 10 days. The drug was most local

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# V. MALARIA

characterized by frequent true relapses beginning as early after the termination of a full course of quinine therapy.

The 30 volunteer subjects were distributed at random into equal groups as shown in table 5. The drugs were administered in single doses of 0.25 gram once weekly for a total of five doses. Malaria was induced by the bites of *A. quadrimaculatus* mosquitoes on the first, third, and fifth days of the second week of drug administration. Subsequent to biting, the salivary glands of the mosquitoes were dissected out, examined for the presence of sporozoites, and positive glands graded on the basis of 1 to 4 plus. The total density of sporozoites in the glands of the mosquitoes biting each subject ranged from 24 plus to 56 plus with an average of 41 plus. Thick blood smears were examined daily until the appearance of parasites. Blood samples

TABLE 5.—Comparison of suppression by weekly doses of 0.25 gm. of santochin, chloroquine, and oxychloroquine in mosquito-induced vivax malaria (Chesapeake Bay area)

| Average inoculum<br>Ratio of patient infections to number exposed.<br>Appearance of parasites<br>Days after first inoculation<br>Range<br>Average<br>Days after last dose of drug | Santochin<br>SN-6911 | Chloroquine<br>SN-7618 | Oxychloroquine<br>SN-8137 |
|---|----------------------|------------------------|---------------------------|
|   | 43+<br>870           | 41+<br>10/10           | 39+<br>10/10              |
|   | 24-33<br>29<br>18    | 43-54<br>50<br>36      | 26-33<br>28<br>15         |

for the estimation of plasma drug concentrations were obtained before and 4 hours after each drug dose and on the day parasites first appeared in the blood.

Transient minimal parasitemia without fever was demonstrated by the thick smear technique in three subjects in the santochin (SN-6911) group and in three subjects in the oxychloroquine (SN-8137) group between the twelfth and fifteenth days after the first inoculation. Thick blood smears failed to reveal parasitemia in any subject receiving chloroquine (SN-7618). However, when a suitable technique for the concentration of parasitized erythrocytes was applied to blood samples obtained during this period, circulating parasites could be demonstrated in all of the subjects in the three groups (7, 8), except for the one volunteer who never developed clinical malaria. It is presumed that inoculation had been unsatisfactory in this case. The shortest prepatent periods (ignoring the transient parasitemias discussed above) in the santochin and oxychloroquine groups were 24 and 26 days respectively. With both these drugs, parasitemia occurred consistently within 33 days, the average being 29 days. In the chloroquine group the shortest prepatent period was 43 days and the average 50 days. In relation to the last dose of drug, the mean para-

On the basis of both total oral dosage and effective mean plasma drug concentration, chloroquine was approximately twice as active as quinacrine in these two malarias. The total dose of chloroquine required to eradicate the erythrocytic phase of McCoy vivax malaria was less than the daily dose of quinine necessary to achieve the same end. The Costa strain of *P. falciparum* was so resistant to the action of quinine that 6 days of therapy with maximum tolerated dosage did not consistently achieve permanent eradication of the erythrocytic trophozoites, while a total dose of 0.65 gram chloroquine did so.

It should be stressed that chloroquine is not a prophylactic nor curative agent in vivax malaria. Fairley (8) demonstrated by subinoculation techniques that even large continued dosage with the drug does not prevent the initial parasitemia of vivax malaria following the bites of infected mosquitoes. Courtney and his colleagues (15) and Alving and his co-workers (16) demonstrated that large daily doses (500 milligrams daily) did not cure the established sporozoite induced vivax infection, although it readily prevented the development of symptoms during the period that drug was present in the tissues.

Extensive trial  
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attack of Southwest Pacific vivax malaria is the most complete to date. Two hundred ninety three delayed primary attacks and first relapses of malaria were treated with three different but adequate dosage regimens of chloroquine. These results were compared to the

quinine respectively. Fever persisted beyond the first day of therapy in only 2.1 percent of the chloroquine patients, but in 8.0 and 8.7 percent of the quinacrine and quinine patients. By 50 days after stopping

chloroquine, 50 days for quinacrine, and 24 days for quinine.

*Clinical use*—Based on experimental evidence summarized above, and on experience derived from extensive investigations in service installations during the war, the Board for the Coordination of Malarial Studies recommended chloroquine dosage regimens (14) that have

ed with

one half the chloroquine in the plasma is bound on the nondiffusible constituents of this fluid

*Toxicity*—The general character of toxic reactions to be expected from chloroquine was indicated above in the comparative study on the effects of large daily oral doses of 4 aminoquinoline derivatives given to young adult subjects. These reactions, difficulty in visual accommodation and pruritus, were observed in some individuals when

to 20

s with

malaria. There were no major signs of toxicity, and in no instance was therapy interrupted. Mild nausea, a rare symptom, generally occurred in fasting patients. Rarely was dizziness noted and there was no tinnitus. Fifty six, or 20 percent of 284 patients carefully observed, complained of pruritus, which was occasionally generalized but usually limited to the palms and soles and was of mild and transitory character. Seven of these patients, or 2.4 percent of the total,

months of therapy in 1 subject. This subsided within 10 days of stopping therapy.

In a statement (14) published in 1946 the Board for the Coordination of Malarial Studies reviewed the toxicity of chloroquine (SN-7618). They state that there are only minor differences between toxicity of chloroquine and quinacrine in a variety of experimental animals. In man, symptoms that may occur during the administration of adequate therapeutic doses of chloroquine include mild and transient headache, visual disturbances, pruritus, and gastrointestinal complaints. The board reviewed the records of approximately 5,000 individuals who had received chloroquine. Every symptom that was observed was recorded in an effort to bring out even minimal toxic

*Antimalarial activity*—The antimalarial activity of chloroquine has already been compared to that of a number of other derivatives of 4 aminoquinoline. Its activity was also compared to quinine and quinacrine, utilizing the previously described standard procedures in McCoy strain vivax infections and in Costa strain falciparum malaria

PENTAQUINE (SN-13,276) AND ISOPENTAQUINE (SN-13,274),  
THERAPEUTIC AGENTS EFFECTIVE IN REDUCING  
RELAPSE RATE IN VIVAX MALARIA

ALF S. ALVING, M. D., *Professor of Medicine, The Malarial Research  
Unit, University of Chicago, Chicago, Ill*

ston (2) of a primary tissue phase of the parasites in certain avian

oriented in 1944 to the search for curative drugs amongst the group of  
compounds known as 8-aminoquinolines. Earlier studies by Sinton  
(3) and James (4) had shown that one compound of this chemical

induced infections

These findings were confirmed and expanded by British (5, 6) and  
American (7) investigators during the war. It is now established that  
pamaquin, administered alone, has moderate curative (8, 9, 10) and  
prophylactic effect (11, 12) in several strains of vivax malaria.  
Quinine potentiates the curative effect of pamaquin. When pamaquin  
is administered during delayed primary attacks or late relapses in

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See footnote at end of next page



quinolines are likewise impractical for the chronic suppression of malaria,<sup>2</sup> but they may have usefulness in the elimination of gametocytes from the blood of patients with malaria (particularly in infections due to *Plasmodium falciparum*) when administered intermit-

malaria

#### CURATIVE EFFECT OF PENTAQUINE AND ISOPENTAQUINE IN VIVAX MALARIA

The curative properties of pentaquine and isopentaquine have been most extensively studied in standardized sporozoite induced vivax infections (21, 22, 23) at the Illinois State Penitentiary (Stateville), which is located in a nonendemic area.

In these investigations (21), healthy, presumably susceptible white volunteers were heavily infected with Southwest Pacific vivax malaria (Chesson strain) (25), usually by the bites of 10 *Anopheles quadrimaculatus* mosquitoes. This strain of malaria is characterized by high relapse rate after suppressive therapy, by a short period of latency between successive attacks, and by almost complete absence of delayed primary attacks. Drug testing was restricted to individuals undergoing primary attacks and first or second relapses, and treatment was initiated promptly after appearance of fever and parasitemia, in order to minimize the effect of acquired immunity.

Under the conditions of these investigations, the relapse rate after treatment with suppressive drugs in patients who had prepatent periods of less than 15 days or latent intervals previous to therapy of less than 30 days was 98 percent. Individuals fulfilling these criteria were, therefore, considered to present a very severe challenge to potentially curative agents. When the prepatent periods or latent intervals were longer, the relapse rate after treatment with suppressive drugs was 67 percent. Such patients were found to present a more moderate challenge to curative drugs (26).

In Table 1 are presented preliminary data on the relative effectiveness of pentaquine and isopentaquine as curative agents when administered concurrently with 2 grams of quinine sulfate daily for a period of 2 weeks to subjects presenting a severe challenge. Results

lapses, Berliner and his co workers found it necessary to administer 90 milligrams of pamaquin daily on a similar regimen to achieve radical cure (8). Shorter periods of administration or lower dosages cure only a fraction of infections induced by heavy sporozoite inocula.

The chief toxic manifestations of pamaquin therapy are upper abdominal and precordial pain, nausea, vomiting, methemoglobinemia, and neutropenia. These symptoms are very severe at the dosage regi-

acute intravascular hemolysis, fortunately occurs rarely in white subjects but occurs in about 5 percent of Negroes treated with pamaquin at daily doses of 30 milligrams or more (15-17). The hemolytic anemia is accompanied by hemoglobinuria and, when severe, by shock. It usually begins on the first day of therapy and may become severe enough to necessitate discontinuance of therapy.

Several hundred 8-aminoquinolines have now been tested for thera-

have participated, two analogues of pamaquin possessing curative effect and less toxicity than the earlier drug have been developed. These are pentaquine<sup>1</sup> (SN-13, 276), 8-(5-isopropylaminoamylamino)-6-methoxyquinoline, and isopentaquine<sup>1</sup> (SN-13, 274), 8-(4-isopropylamino-1-methylbutylamino)-6-methoxyquinoline.

#### PROPHYLACTIC AND SUPPRESSIVE EFFECT OF PENTAQUINE AND ISOPENTAQUINE IN VIVAX MALARIA

Both pentaquine and isopentaquine prevent the development of malaria if they are administered for 8 days, beginning the day before

<sup>1</sup> Pentaquine was first synthesized by Dr. Nathan Drake, Department of Chemistry, University of California, San Diego.





pentaquine 9 out of 34 or 26 percent, while 6 out of 15 or 40 percent of those receiving pamaquin relapsed<sup>4</sup>

It is also apparent that even on low dosage regimens these three 8 aminoquinolines exert definite but slight effect on the immediate relapse rate after treatment. The curative properties of the drugs are demonstrated, however, not only by their immediate effect on the relapse ratio, but also by their effect on the subsequent latent intervals and on the total number of relapses. For example, even in dosages as low as 30 milligrams per day, pentaquine, administered with quinine, prolonged the following latent interval markedly in about half the

the drugs reduce the number of persisting tissue phases of the malarial parasites

The curative action of pentaquine is enhanced by quinine, as is also that of pamaquin. For example, the relapse rate in severely infected patients was 50 percent when pentaquine was administered alone at 60 milligrams daily, while it was only half as great when 2 grams of quinine sulfate were administered concurrently. The curative action is not potentiated by quinacrine, chloroquine, chloroguanide (paludrine), metachloridine, or sulfadiazine in heavy experimental infections (19). Other cinchona alkaloids have less potentiating effect than quinine (20).

The above results pertain to Chesson strain virax infections in which the relapse rate after quinine would have been practically 100 percent. In more moderate infections, that is, those in which the relapse rate would have been 67 percent after treatment with suppressive drugs, only 2 out of 45 patients or 4 percent relapsed after treatment with 60 milligrams of pentaquine administered concurrently with quinine (table 1).

Although the series is too small to define precisely the relapse rate on

and other characteristics of the strain of malaria under consideration, the density of the sporozoite inoculum, the degree of natural or acquired immunity of the host, and possibly antiparasitic factors due to previous therapy

emia, which can, however, be abolished by the concurrent oral administration of 0.5 gram of methylene blue daily in divided doses. The margin between the effective therapeutic dose and the maximum tolerated dose for isopentaquine is, therefore, much greater than for either pentaquine or pamaquin, being fourfold in severe experimental infections. Isopentaquine has not been tested in naturally acquired vivax malaria at either intermediate or low dosages, but it seems safe to predict that, in treatment of late clinical attacks, the factor of safety will be of the order of magnitude of 8 or 10.

### CONCLUSIONS

The chief value of pentaquine and isopentaquine as therapeutic agents is in the treatment of late clinical attacks of vivax malaria. In very heavily infected nonimmune adult individuals (table 2)

TABLE 2.—Recommended curative therapy in vivax malaria

|               | Primary attacks <sup>1</sup>                                     | Relapses <sup>2</sup>   |
|---------------|--|---|
| pentaquine    | 60-mg. base with 20 gm. quinine sulfate <sup>3</sup> for 14 days | 30-30 mg. base with 20 gm. quinine sulfate <sup>3</sup> for 14 days |
| isopentaquine | 60-mg. base with 20 gm. quinine sulfate <sup>3</sup> for 11 days | 30-mg. base with 20 gm. quinine sulfate <sup>3</sup> for 11 days    |

<sup>1</sup> Drugs should be administered every 4 hours throughout the 24 hours.

<sup>2</sup> Drugs may be administered on a b. i. d. schedule.

<sup>3</sup> The minimum daily dose of quinine necessary to achieve potentiation has not been determined.

treated during primary attacks or early relapses, it is necessary to administer 10 milligrams of pentaquine or isopentaquine and 0.33 gram of quinine sulfate every 4 hours throughout the 24 hours for 4 days. Because of the possible occurrence of acute hemolytic

For the treatment of less heavily infected or partially immune individuals, the total daily dose of pentaquine or isopentaquine may be divided. Drugs may be administered three times a day while the patients remain ambulatory but under medical supervision.

Isopentaquine is superior to both pentaquine and pamaquin. It has a slightly greater curative effect on an equal weight basis, but its chief advantage is the greater margin between the therapeutic and toxic dose.

In addition to the curative properties in vivax malaria, these drugs used intermittently in nontoxic dosages may have value as gametocidal agents, particularly in falciparum infections where gametocytes, once they have developed, are resistant to the action of most antimalarial agents. The elimination of gametocytes from the blood

not been noted in white subjects during the administration of isopentaquine, but less than 100 patients have been treated at the maximum therapeutic dose. Both of the new drugs probably are less dangerous from this standpoint than pamaquin. This is suggested by the fact that one patient who developed an acute hemolytic crisis during treatment during a later relapse without recurrence of the

The comparative toxicity of pentaquine and isopentaquine in

the point where final conclusions may be drawn as to the relative frequency of hemolytic crises. Preliminary observations, however, suggest that the two drugs are of about equal toxicity and may have a somewhat greater tendency to produce hemolytic anemia in this group than they do in white subjects. It would seem reasonable to expect that much smaller dosages of drug could eradicate vivax infections in

usefulness in tropical areas.

maximum tolerated dose. Pamaquin causes complete eradication of vivax malaria in experimental infections presenting a severe challenge to the drug only when administered in the maximum tolerated dose of 90 milligrams, or 200 milligrams of its naphthoate salt, daily. Even in the treatment of late relapses, the dosage of pamaquin necessary to achieve cure in a high percentage of individuals allows only a threefold margin of safety.

The maximum tolerated dose of pentaquine is 120 milligrams per day. General symptoms at this dosage are extremely severe, about equal to the toxicity of 90 milligrams of pamaquin, but the spectacular symptom of postural hypotension, which has not been observed with either pamaquin or isopentaquine, occurs frequently. Nevertheless pentaquine has a greater margin between the therapeutic and toxic dose than has pamaquin. In severely infected nonimmune individuals, it is about twofold, in patients having considerable immunity, it is about fourfold.

complication noted at this high dosage has been marked methemoglobin

# PALUDRINE IN THE TREATMENT AND CONTROL OF MALARIA

B. G. MAEGRAITH, *Liverpool School of Tropical Medicine*

The discovery of paludrine arose from the study by Curd,

antagonism to essential enzyme systems of the parasites, and a long series of experiments was carried out on these lines before a successful compound was synthesized. As the work progressed, it became apparent that the intact pyrimidine ring was not essential for antimalarial activity, and eventually the carbon atoms in the five and six positions were omitted. The skeleton remaining was related to the biguanide system and used for subsequent synthesis. Eventually the compounds 4430 and 4688 were synthesized and proved to be extremely active in bird and human malaria. The more effective and less toxic of the two compounds, 4688, was tested extensively and finally established as an effective antimalarial in human infections. It was subsequently called paludrine.

Diseases Center, Liverpool, by the staff of the School of Tropical Medicine

drine) showed that both were very active. Clinical trials were continued in Liverpool and at this stage the investigation of the suppressive properties of these drugs and their mode of action was taken over by a group of workers in Australia. The results of the research referred to and of subsequent extensive trials have established paludrine as an antimalarial drug of great potency.

## PALUDRINE AS A THERAPEUTIC AGENT

Our investigations were designed to determine the therapeutic range of activity of paludrine and its possible toxic effects. It was found



not yet clear whether intravenous paludrine is as effective in complicated malignant tertian cases as intravenous quinine, but its point of action (on the dividing stages of the parasite) indicates that in infections where trophozoites predominate, quinine may be more efficacious and more rapidly active. Controlled experiments on this point are at present being carried out. There is no contraindication for giving both quinine and paludrine simultaneously, there are, in fact, advantages to be seen in this technique in pernicious cases since quinine acts on the parasite at an earlier stage in the asexual cycle than paludrine.

*Relapses of P. vivax malaria*—The effect of paludrine dosage on the subsequent relapse rate of *P. vivax* malaria has been studied by several workers. For instance, we compared the effect of dosage regimes of 50 and 500 milligrams paludrine twice daily for 14 days with mepacrine

other dosage regimes were obtained by Johnstone. It was clear, therefore, that paludrine administered in this way is not effective in reducing the relapse rate and cannot compare with the combined treatment with quinine and pamaquin developed in India by Sinton and used with good success in the 1939-45 war (Kelleher and Thompson, 1945).

The action on *vivax* relapse rates of long continued administration of paludrine has also been followed. Patients treated with single

pamaquin. Pamaquin toxicity is, however, likely to be exhibited. Since the relapse rate can be considerably reduced by this means, it is probably wise to treat all cases of *P. vivax* malaria with a combined course, rather than take advantage of the remarkable action of a single dose on the clinical attack. The time saving value of the latter treatment is, however, obvious in dealing with attacks in the indigenous populations of endemic areas.

Cases treated with single doses of paludrine and then given no further treatment relapse after a certain period, varying from 10 days to 6 weeks.

*Relapses of malignant tertian malaria*—The experience of other workers confirms the experimental work of Fairley in 1946, who showed

*Treatment of the acute attack of P. vivax malaria*—In 1945 we treated over 150 cases of naturally acquired *P. vivax* infections with oral paludrine in doses ranging from 5 milligrams to 750 milligrams given twice daily for 14 to 28 days. Cure of the clinical attack was obtained in all cases. Doses as low as 25 milligrams given twice daily produced clinical cure in some cases. At dosages of 500 milligrams or more twice daily, patients occasionally complained of nausea and sometimes vomited, but these were the only toxic effects observed, it was never necessary to terminate treatment because of them. The results of treatment were so encouraging that it was decided to reduce the total dosage substantially. It was found that the clinical relief of the attack could consistently be obtained by the administration of single doses of 500 milligrams given twice daily for 7 to 10 days upwards. This dosage was found to be effective in preventing relapse.

Adams et al (1945) found that paludrine brings about clinical cure and the disappearance of trophozoites from the peripheral blood at a dose of 500 milligrams given twice daily for 7 to 10 days.

*Treatment of the acute attack of P. falciparum malaria*—We investigated the therapeutic activity of paludrine in primary cases of *P. falciparum* infection, using in the first instance the same dosage as for *P. vivax* infections. Oral doses of 50 to 600 milligrams paludrine given twice daily for 7 to 10 days.

Such dosage has been tried experimentally by other workers, who have found that the uncomplicated case will often respond readily. Failures have been reported on these low dosages, however, so they cannot be regarded as a practicable proposition. It is essential in dealing with malignant tertian malaria to make sure that the patient receives adequate dosage. For this reason in Liverpool the standard treatment adopted at present is 300 milligrams paludrine twice a day for 7 to 10 days. In severe cases, 500 to 600 milligrams given twice daily for 7 to 10 days.

Paludrine does not settle for 3 or 4 days after the commencement of treatment, though parasites are usually absent from the blood by the end of the third day.

*Parenteral administration*.—Paludrine can be administered intravenously without ill effect to the patient. Doses of up to 150 milligrams may be given at a single injection and repeated after 4 hours. It is



*The mode of action of paludrine*—By means of serial subinoculation of blood from infected subjects to volunteers (subinoculation) Fairley and his colleagues demonstrated that paludrine acts differently from mepacrine and certain other antimalarial drugs. They deduced from their experimental results that the primary wave of erythrocytic parasites arising from the preerythrocytic forms of the plasmodia is inhibited in both *P falciparum* and *P vivax* infections. In *P falciparum* infections the preerythrocytic forms are destroyed. In *P vivax* infections they are incompletely destroyed, but their development is delayed, some survive and eventually give rise to overt malaria. The action of paludrine in *P falciparum* infections is thus that of a causal prophylactic. It has no such effect in *P vivax* malaria. By timing the dosage in relation to the development of the parasites other experiments showed that paludrine acts in the early stages of *P falciparum* malaria on the preerythrocytic forms, and possibly on the sporozoites, although the latter action is uncertain.

Black has recently shown that in vitro paludrine inhibits the development of *P falciparum* beyond the stage of the early schizont. It therefore acts later in the asexual cycle than quinine or mepacrine,

explanation of its action on the developing gametocytes in the mosquito

Theories concerning the action of the drug have been advanced by several authors, but so relation of the chemi cates possible activity

in which such substances as adenylic acid, adenosine etc., are important. It is possible, for instance, that some form of competition exists between the drug and adenosine in the synthesis of the di- and tri-phosphonucleotides or in relation to the activity of the adenosinases of tissue or plasma.

Recently Curd and Rose (1946) pointed out the similarity between the metallic complexes which paludrine can form and the metal protoporphyrins. They suggested that paludrine may interfere with some porphyrin system specific to the parasite.

*Acquired resistance to paludrine in plasmodium gallinaceum.*—Lourie and his colleagues (1947) studied the production of the development of drug resistance in strains of *P gallinaceum* in chicks. The hydrochloride mepacrine, a forerunner of paludrine, showed a high degree of resistance, in serially subinoculated strain in one series the other drugs but was e, the pyrimidine ring is

clearly that adequate treatment with paludrine sterilized most malignant tertian infections. It is not yet known whether occasional naturally occurring paludrine resistant strains of parasites exist, but there is evidence from West Africa pointing in this direction.

#### PROPHYLAXIS AND SUPPRESSION OF MALARIA WITH PALUDRINE

Fairley and his colleagues in Australia have shown that paludrine is a causal prophylactic in sporozoite induced *P. falciparum* malaria in the sense that it deals with pre-erythrocytic forms. In Fairley's ex-

periment, the infection became overt after a period. In field experiments it was found that volunteers taking 100 milligrams daily and subjected to continual biting by heavily infected mosquitoes did not develop either *P. falciparum* or *P. vivax* malaria. During these experiments the volunteers were subjected to extremes of exercise, heat, and cold, as well as to injections of adrenalin and insulin, but parasites did not appear in the blood while they were taking the drug. About 3 to 5 weeks after the volunteers ceased to take the drug, *vivax* infections became overt, *P. falciparum* infections were cured.

The most suitable dosage for prophylaxis and suppression in the field has not yet been determined. Causal prophylaxis in *P. falciparum* infections has been obtained experimentally with a dosage regime of 100 milligrams taken once weekly. On this dosage most cases of *P. vivax* infections may also be suppressed, but an occasional break

occurs. When dealing with individuals already heavily infected with *falciparum* malaria, full therapeutic dosage is necessary before prophylaxis is begun.

#### ACTION OF PALUDRINE ON GAMETOCYTES

Fairley (1916) found that daily doses of 100 to 300 milligrams paludrine had no apparent effect on *P. vivax* or *P. falciparum* gametocytes.

Gametocytes, however, ceased at an early stage in the mosquito in the presence of paludrine. The drug is thus more effective from this point of view than either quinine or mepacrine.



absent Resistance to paludrine persisted after passage through *Aedes aegypti*

The significance of these observations in relation to human malaria is obvious, and is being investigated

### THE HUMAN PHARMACOLOGY OF PALUDRINE

**Toxicity**—In animals the toxicity of paludrine is low One of its great virtues is its freedom from toxic effects over a very wide range of therapeutic activity No ill effects have been noted with therapeutic regimes of less than 1,000 milligrams per day At this dose and up to 1,500 milligrams per day some subjects, but not all, complain of epigastric discomfort and nausea and may vomit No other side effects have been observed in cases treated at Liverpool, but Fairley has reported the presence of red blood cells and hyaline casts in the urine of volunteers on high doses Haematuria has also been reported occasionally in children given high dosages

In suppressive dosage no ill effects have been reported

**Absorption and excretion**—Paludrine is absorbed rapidly After a single dose the plasma concentration reaches a maximum in 2 to 4 hours and falls rapidly with a half-life of about 20 hours

of paludrine in whole blood varies from two to four times that in the plasma

drine present in plasma is protein bound so that the effective concentration in plasma measures roughly a quarter of the total amount present The concentration of paludrine in certain human tissues resembled those observed in animals (Spinks 1947) The drug was most concentrated in the kidney and liver In this respect it differs from mepacrine and 3349 which are most concentrated in the liver and less in the kidney (Army Malaria Research Unit, 1946, Spinks, 1946)

percent of the drug is excreted in the urine After a single dose the

pentaquine was given in amounts of 60 milligrams combined with 2 grams of quinine daily, it was noted that only 3 percent relapsed as compared with 67 percent when quinine or pentaquine was given separately. Abdominal distress was the outstanding toxic symptom, mild anorexia was occasionally encountered, and a few complained of transient weakness, headache, or diarrhea, but these symptoms were never severe. Methemoglobinemia was found in approximately 25 percent of the subjects but was of a mild grade. The toxicity associated with 60 milligrams of pentaquine was approximately equivalent to that observed with 30 milligrams of pamaquin. These studies definitely demonstrated that it was possible to eradicate mosquito induced vivax malaria of the Southwest Pacific variety in the majority of instances. However, the drug had to be given with quinine to secure this effect and the toxicity was sufficient to require hospitalization during the course of the 2 weeks' treatment, primarily for the purpose of close observation of the patients.

#### TREATMENT OF RECURRENT MALARIA IN EX-SERVICEMEN

In view of the encouraging results obtained with pentaquine as a curative compound in the volunteers, it was deemed advisable to determine its effectiveness in patients who had contracted their malaria in various overseas theaters and who were continuing to suffer from relapses at intervals.

In the Army there were 462,080 admissions for malaria from 1942 to 1945 inclusive, with 276 deaths, and in the Navy there were 113,744 admissions with 87 deaths and 3,303,000 sick days. On the same basis the Army probably had to credit 13,000,000 sick days to malaria, making a total of 16,000,000 sick days for the two services. For each case admitted to a hospital or dispensary for treatment, there were many more cases which were self treated and consequently not included in the above figures. The men rapidly became familiar with the pattern of symptoms which initiates an attack and would use quinine to treat the attack as soon as it started.

In cases of vivax malaria, and in some their initial attack dates back to

was necessary to treat them in an ambulatory fashion and with a lower dosage than was used in the original studies of Alving in order to

# THE CURE OF RECURRENT VIVAX MALARIA AND STATUS OF IMMUNITY THEREAFTER<sup>1</sup>

L. T. COGGESHALL, M. D.,<sup>2</sup> FRED A. RICE, M. D.,<sup>3</sup> and  
ERNEST H. YOUNT, JR., M. C.,<sup>4</sup> A. U. S.

Relapsing malaria has complicated therapeutic investigations for many years. In highly endemic areas it is not possible to know whether the presence of an acute infection is a relapse or an initial infection. The results of previous studies have indicated that an attack of malaria confers a relatively low grade immunity once the infection has been eliminated. Thus, a clinical exacerbation following therapy is not necessarily indicative that no cure was obtained. This was found to be true particularly in animals where carefully controlled observations were possible. For example, Maier and Coggeshall (1) found that sulfonamides would eradicate chronic *Plasmodium knowlesi* infections

endemic areas of many thousands of veterans with chronic malaria afforded an unusual opportunity to provide the answers to two ques-

results of such a study form the content of this paper.

As the result of an intensive cooperative investigative program conducted under the auspices of the National Malaria Research Council, a compound was reported (2) which was numbered 13276 and was named aminoamyla-

pound, was first synthesized by Drake (3). In experimental animals, its activity was found to be 80 to 120 times that of quinine and 2 to 8 times that of pamaquin, depending upon the strain of plasmodium

<sup>1</sup>This study was supported jointly by a research grant from the United States Public Health Service and the Department of Medicine, University of Chicago. The drug pamaquin, was supplied by the Abbott Laboratories.

<sup>2</sup>Dean, Division of the Biological Sciences, the University of Chicago.

<sup>3</sup>Assistant Resident, Department of Medicine, the University of Chicago.

<sup>4</sup>Captain, A. U. S., assigned to Illinois State Penitentiary at Joliet, Ill., on the malaria project.

sporozoite and that following trophozoite inoculations are quite different.

For this immunity study in man, volunteers who had been treated and cured by Alving and coworkers were reinoculated after cure. Originally, they had been inoculated with the Chesson strain of South west Pacific malaria, which had been brought to this country and which is characterized by a frequent and predictable relapse rate. The men were selected from 350 white healthy volunteers who gave no previous history of malaria and who were infected with sporozoites by mosquito bite. Thus an accurate malaria history was available for each individual, as the duration and severity of infection prior to cure was known. Since cure was possible, any reactivation of clinical or parasitic activity could be ascribed to reinfection and not to relapse. In this study, attempts were made to evaluate the immune response to homologous sporozoite, as well as homologous and heterologous trophozoite, inoculations following cure.

Twenty one volunteers who had received chemotherapeutic cure by treatment with pentaquine and quinine in amounts sufficient to eliminate all inoculate mosquito group 2,

Chesson strain, and in group 3 3 were injected with trophozoites of the heterologous St. Elizabeth strain of *Plasmodium vivax*. A comparison was made between the initial attack and the final relapse before the curative drugs were used as well as the attack following reinocu-

### SPOROZOITE REINOCULATION

The 10 volunteers in group 1 who were subjected to reinfection by the bite of mosquitoes had experienced from 1 to 7 previous attacks of malaria and from 3 to 53 days of untreated malaria prior to chemotherapeutic cure. The interval between cure and reinoculation varied from 3½ to 21 months. Two had experienced one attack, 2 had had 2, 2 had had 3, and 1 each had had 4, 5, and 7 attacks prior to cure.

With the exception of one individual, there was little evidence of resistance in those who had experienced fewer than four previous

decrease greatly the possibilities of toxic reactions. It must be emphasized that the early cures obtained in volunteers followed treatment on the third or fourth day of the initial attack, and, as such, the patients had acquired little or no specific immunity. The ex-servicemen, with their repeated attacks, presented a possibility of combining the therapeutic effectiveness of the drugs with a highly active humoral and cellular defensive mechanism. In July 1946, the

should establish a point inferential in this paper, regarding the possibility that some of these cases which have relapsed for a long time can be cured with chloroquine alone. At the outset, the men were observed daily, with blood counts and urine examinations. No hospitalization was required.

During this 2 year period of observation, arrangements were made to treat approximately 400 ex-servicemen. In order to reduce the pos-

lause has occurred and therefore a statement is made.

The toxic reactions were minimal and for the most part were attributable to the quinine, although two cases of temporary postural hypotension were found. There was no conclusive evidence that the kidney or hemopoietic system had been damaged.

#### PRESENCE AND DURATION OF IMMUNITY FOLLOWING CURE

It can readily be seen that with the data available there is available observations to the type of immunity from that dependent upon a subclinical and undetected infection.

As stated above, the immunity following eradication of monkey malaria parasites by the sulfonamides was of relatively short duration. However, the behavior of the strain of parasite used was dissimilar to that of the



## HOMOLOGOUS STRAIN TROPHOZOITE REINFECTION

The eight volunteers in group 2, previously cured of their mosquito induced malaria, were each reinoculated by the intravenous injection of whole blood containing 500,000 homologous trophozoites of the Chesson strain of *Plasmodium vivax* malaria. They were divided into two groups according to the length of the interval after cure before reinoculation. Four had had, prior to reinoculation, from one to four clinical attacks and from 1 to 15 days of untreated malaria. The interval between chemotherapeutic cure and reinfection ranged from 7 to 19 months. The second group of four had had one to five prior relapses but a shorter interval after cure before reinoculation—namely, 20, 37, 60, and 60 days. Three of the four men who were reinoculated with trophozoites within 7 to 19 months after their cure had acute clinical attacks. One was resistant to reinfection. Of the four who were reinoculated on the twentieth to the sixtieth day following cure, all were highly susceptible and experienced parasitemia and a clinical course comparable to that observed in their initial attack.

## HETEROLOGOUS STRAIN REINFECTIONS WITH TROPHOZOITES

Three of the four men who had been infected with the American St. Elizabeth strain of *P. vivax* malaria. Two of the men had had three attacks of the Southwest Pacific malaria, and the intervals between therapy and reinfection were 13 and 18 months respectively, while the third had experienced eight attacks, and 1 month following cure he was reinoculated. In each instance, the three men developed a course of malaria which, as far as the observation period extended, resembled that of a nonimmune individual.

## SUMMARY

1. Pentaquine, when given under the dosage regime of 30 milligrams per day for 2 weeks in combination with quinine 2 grams daily

therapy cannot be completely ruled out, although the above numbers are statistically significant and thus would not seem to be mere coincidence.

## V. MALARIA

density of parasites necessary to produce a fever of 103° F. The one exception was in a volunteer who had had only three attacks of malaria prior to reinfection, but he had had malaria for a total of 53 days. An increase in his pyrexia old for 103° F rectally, from 10/cmm with his primary 1,890/cmm following reinfection was observed.

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greater than 103° F rectally during their last relapse, with  
of malaise, nausea, myalgia, and headache. When  
fection, all three were sufficient  
be sufficient  
effect was

1  
2  
from 24 to 36 hours duration of

was less resistant to reinfection after 53 days of malaria and only three attacks, than two others who had had 23 days of malaria but more than four attacks.

Interval between chemotherapeutic cure and reinfection ranged from 3½ to 20 months, however, greater sampling with a better distribution would have been advantageous. It is concluded that the results of the study are re-  
fer

# NOUVEAUX MÉDICAMENTS DU PALUDISME—ÉTUDE COMPARÉE DE LEUR ACTIVITÉ DANS LE TRAITEMENT CURATIF ET EN PROPHYLAXIE

J SCHNEIDER, PH DECOURT, et D MICHAIL, *Faculté de Médecine de Paris, Paris, France*

De 1941 à 1948, nous avons étudié de nouveaux antipaludiques de synthèse

Les résultats que nous rapportons résument notre opinion sur leur valeur comparée dans le traitement curatif du paludisme

En prophylaxie nous apportons une première série de résultats, des expériences complémentaires sont en cours.

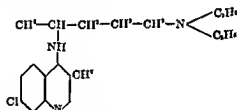
Ces expériences ont été faites en collaboration avec les Services de la Santé Publique de Tunisie et du Maroc

## I TRAITEMENT CURATIF

Nos résultats sont valables pour le traitement de l'accès de paludisme, nous ne porterons pas encore de jugement sur l'activité comparée des nouveaux médicaments dans la prévention des rechutes un recul de plusieurs mois nous est encore nécessaire

Nous avons étudié

(1<sup>o</sup>) la *méthyl-3 (diéthylaminopentyl) amino 4 chloro 7 quinoleine* (Sontoquine ou Nivaquine C-M-R)



Les premiers essais ont été faits en 1941, de 1941 à 1943, trois sels ont été expérimentés

supérieure aux précédents

Dans une première série, 53 cas furent traités avec Og 30 par jour pendant 5 jours les résultats furent supérieurs à ceux obtenus avec les mêmes doses de Quinacrine

4. The degree of immunity after infection with homologous strain  
 protozoites bore a positive relation to the number of relapses but not  
 the duration of malaria prior to cure

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ate reinoculations

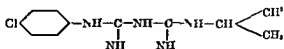
6. Finally, as a broad generalization, these studies indicate that the  
 duration of immunity following cure is variable but in general rela-  
 tively mild and of short duration

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Les deux dérivés, de formule très voisine (différentes par un radical  $\text{CH}_3$  sur le noyau), ont une activité comparable. Sa plus grande facilité de fabrication nous a fait préférer l'emploi du dérivé non méthylé.

(3°) le *N*<sup>1</sup> *p* chlorophenyl *N*<sup>2</sup> isopropyl biguanide (spécialisé par l'I.C.I. sous le nom de Paludrine)



Nous avons employé le dichlorhydrate

Les publications britanniques proposaient des posologies variables oscillant entre 0g 10 et 1 gr par jour pendant des périodes de 1 à 14 jours, et plus.

Nos essais nous ont montré qu'une dose unique de 0g 10 proposée par certains pour juguler une crise de paludisme, n'est habituellement pas suffisante.

Par ailleurs, nous avons constaté qu'une dose quotidienne de 0g 30

“

,

Nos premiers résultats furent les suivants

—*P. falciparum*—sur 30 malades, la durée moyenne de l'état fébrile était de 1 jour, 77 et les schizontes disparaissaient après 2 jours 4

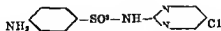
—*P. vivax*—sur 30 malades la durée moyenne de l'état fébrile était de 2 jours 77 et la durée moyenne de persistance des schizontes de 3 jours 88

—*P. malariae*—sur 15 malades, la durée de l'état fébrile était de 2 15  
*iparum*, mais

in ~  
 (4°) Sulfamides

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L<sub>1</sub>  
 espoir  
 (a)  
 chloridine)

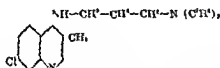


Lorsque l'expérimentation commença, nous n'avions aucun élément concernant les doses pour l'homme, les résultats remarquables ob

Nous avons retenu ce sel en utilisant les doses suivantes : 0g 60 le 1er jour, 0g 50 le 2<sup>e</sup> jour et 0g 30 pendant les trois derniers jours du traitement.

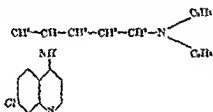
Ainsi, chez plus de 400 malades (F. V. M.), nous avons obtenu l'apyrexie en 36 à 40 heures et la disparition des schizontes en 60 à 65 heures.

*Méline* (Brachysan).



Le dibromhydrate aux mêmes doses que la Nivaquine C est bien moins actif; nous l'avons abandonné.

(b) le (diéthylaminopentyl) amino-4 chloro 7 quinolène  
(Résoquine—Nivaquine B—Chloroquine)



Deux sels ont été étudiés

—le sulfate

—le diphosphate

inférieurs sur *P. falciparum*, légèrement supérieurs sur *P. vivax* et identiques sur *P. malariae*.

## ACTIVITÉ COMPARÉE

Nous nous sommes efforcés de préciser leur activité comparée sur le traitement d'accès du paludisme chez des malades admis dans les mêmes conditions dans les hôpitaux indigènes de Fes et de Tunis.

Les médicaments, aux doses exprimées ci-dessus, étaient répartis en deux prises par jour, la température contrôlée deux fois par jour et reprise en cas d'accès. Un contrôle parasitologique (goutte épaisse) était fait deux fois par jour.

Les résultats rapportés dans la table ci-dessous portent sur 250 malades.

la persistance des parasites

TABLEAU I

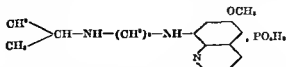
|  | Durée de la fièvre |      |      | Durée de persistance des schizontes |      |      |
|--|--------------------|------|------|-------------------------------------|------|------|
|  | Fal                | Vir  | Mal  | Fal                                 | Vir  | Mal  |
| (1) méthyl-3 (diéthylaminopentyl) amino-4 chloro-7 | 1 57               | 1 70 | 1 65 | 2 5                                 | 2 20 | 2 8  |
| " " " " " "  | 1 70               | 1 62 | 1 6  | 2 75                                | 2 20 | 2 3  |
| " " " " " "  | 1 77               | 2 77 | 2 66 | 2 40                                | 2 65 | 7 15 |

Nos conclusions sont que, malgré les résultats observés dans le paludisme aviaire indiquant une supériorité presque double de la nivaquine B, résoquine, chloroquine sur la sontoquine, les résultats cliniques sont comparables.

Tous deux sont supérieurs au N<sup>o</sup> p chlorophényl N<sup>o</sup>-isopropyl biguanide (Paludine) en ce qui concerne *P. vivax* et *P. malariae*, aussi nous estimons que, pour le traitement curatif du paludisme, la nivaquine est le meilleur médicament actuel.

(5°) En dehors de ces différents schizonticides, nous avons étudié un nouveau gaméticide.

(isopropylamino-5'n amylamino)-8 méthoxy 6 quinolène monophosphate (Pentaquine).



L'expérimentation avait montré une toxicité deux fois moindre que celle de la Plasmoquine (Praéquine) et une activité sur *P. gallinaceum* environ deux fois supérieure.

L'expérimentation clinique ne nous autorisa pas à tirer les mêmes conclusions administrées à la dose de 0,3 pendant 3 ou 5 jours, la

tenus sur *P. gallinaceum* nous avaient incités à l'essayer bien qu'il fut peu actif sur *P. praecox* (canari).

Plusieurs groupes de malades furent étudiés

Un premier essai de 6 malades traités à la dose quotidienne de 0g 50 fut un échec

Un deuxième groupe de 8 malades traités avec 1 gr par jour fut un échec partiel

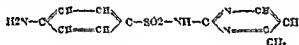
Un troisième de 4 (2 à *P. falciparum*, 2 à *P. vivax*) à la dose de 2 gr par jour la guérison ne fut obtenue qu'au 5<sup>e</sup> jour pour *P. vivax* et qu'au 3<sup>e</sup> jour pour *P. falciparum*

Un quatrième de 6 (2 à *P. falciparum*, 1 à *P. vivax*) et 3 formes mixtes *P. falciparum* + *P. falciparum* + *P. vivax* fut traité à la dose de 4 gr par jour l'apyrexie et la disparition des schizontes furent obtenues après le 3<sup>e</sup> jour pour *P. falciparum* et après le 5<sup>e</sup> jour pour *P. vivax*

Enfin, deux malades furent traités avec 6 gr par jour (1 *P. falciparum*, 1 *P. vivax*) dans les deux cas l'apyrexie fut obtenue en 48 heures, les schizontes disparurent le 3<sup>e</sup> jour pour *P. falciparum* et le 4<sup>e</sup> pour *P. vivax*

Jusqu'à 4 gr la tolérance était bonne, pour les malades traités avec 6 gr on constata des signes d'intolérance nous obligeant à nous limiter à cette dose maxima

(b) la sulfaméthylidiazine (sulfamérazine, Sumédine)



suite de rechûtes dès le 18<sup>e</sup> jour

Pour 8 cas de *P. vivax* traités avec 4 gr par jour, on observa deux cas favorables, pour les 6 autres il fallut faire un autre traitement.

Comme pour la Mischloridine, l'activité est moindre pour *P. vivax* que pour *P. falciparum*.

En conclusion, les sulfamides actuellement connus ne présentent

les trois variétés de plasmodium

On a vu que la quinine (quinine) et paludrine qui sont le mieux tolérées aux doses thérapeutiques sur plus de 1 000 malades nous n'avons pas noté de cas notables d'intolérance



à la même dose hebdomadaire de Og 30. Les résultats ont été rapportés dans une précédente communication dont voici le résumé  
—Nombre total de sujets: 2 005.

—1 120 traités

—885 témoins

Les index spléniques et plasmodiques successifs indiquent une diminution de l'impaludation dans les secteurs traités

Les résultats obtenus sont les suivants :

ramener les index plasmodiques à 0

Le nombre de cas de paludisme a été ramené à 0 dans les secteurs traités

1° En Tunisie

Maroc et en Tunisie. Notre but était de comparer dans les mêmes conditions par rapport à des témoins non traités, l'activité des nouveaux dérivés et celle de la Prémaline que, de longue date dans les divers pays de l'Union française, on emploie en prophylaxie collective<sup>1</sup>.

Des campagnes de prophylaxie, portant sur des collectivités de plusieurs milliers de personnes au Maroc, au Cap Bon tunisien, à Gabès, etc., avaient depuis longtemps prouvé qu'une dose hebdomadaire de 3 comprimés pour les adultes et des doses fractionnées pour les enfants abaissaient considérablement les index spléniques et plasmodiques et

ne) (3359

RP)

(2°) l'association Paludrine + Rodopréquine

(3°) l'association Nivaquine B + Rodopréquine (Prémaline N)

Pour obtenir des résultats comparables, nous avons utilisé les mêmes doses, soit Og 30 de schizonticide + Og 03 de Rodopréquine lorsque celle-ci était associée à l'un des dérivés, le rythme de distribution pour tous les dérivés était hebdomadaire

### (1°) EXPÉRIENCE DU MAROC

Quatre groupes furent choisis pour comparer deux à deux chacun des dérivés

Le tableau 2 résume les résultats

<sup>1</sup> La Prémaline est une association de Og 10 de Quinacrine + Og 01 de Rodopréquine par comprimé

Pentaquine a donné des résultats légèrement inférieurs à ceux obtenus avec les mêmes doses de Rodoprequine<sup>1</sup> et il fallut donner Og 06 par jour pendant 3 ou 5 jours pour obtenir un résultat comparable

Etant donné que les doses quotidiennes de Og 03 de Rodoprequine sont très bien tolérées, nous ne considérons pas la Pentaquine comme supérieure.

## II. ESSAIS DE PROPHYLAXIE

vivant au milieu d'une population non soumise à la prophylaxie

A Prophylaxie collective Nos experiences de prophylaxie collective ont ete faites en Afrique du Nord ou le paludisme dure habituellement du debut Juin a fin Novembre

Dans ces expériences on soumet à la prophylaxie la population

- (1°) Les index spléniques et spléno-métriques } établis pour la totalité  
(2°) Les index plasmodiques } de la population  
(3°) Le dénombrement des cas de paludisme confirme

Trois index sont établis

- 1 au début de la prophylaxie (fin Mai)
- 2 au milieu (mi Août)
- 3 à la fin (fin Novembre)

Nous avons fait quatre séries d'expériences

- I La première pendant l'été 1942, pour comparer la Sontoguiné (Nivaquine M) à la Quinacrine

Les résultats ont été communiqués aux autorités médicales américaines (Alger 1943) et au Congrès International d'Alger (Fev 1944)

Nos conclusions étaient que la Sontoquine était au moins aussi active que la Quinacrine.

- 11 La deuxième pendant l'été 1945 la sécheresse persistant en Afrique du Nord avait réduit l'anophélisme aussi tous les secteurs, je n'étais au début de l'expérience des index faibles, la hausse estivo-automnale du paludisme fut insignifiante et nous décidâmes pour la rigueur de l'expérimentation de ne pas tenir compte des résultats.

- III Une troisième expérience fut faite en 1946 à Ghardimaou en

\* Association à parties égales de Plasmoquine (Praéquine) et de Rhodoquine (710 F)

TABLEAU 2—Continued

|  | Index plasmodiques |       | Index épidémiologiques |       | Index épidémiométriques |        | Acès palustres confirmés |   |
|--|--------------------|-------|------------------------|-------|-------------------------|--------|--------------------------|---|
|  | A                  | E     | A                      | E     | A                       | E      | A                        | E |
| <i>Dar Caid Ahmed I</i>  |                    |       |                        |       |                         |        |                          |   |
| (7 adultes+68 enfants)   |                    |       |                        |       |                         |        |                          |   |
| { Début  | 8 33               | 26 47 | 20 83                  | 60 88 | 29 18                   | 137 49 |                          | 1 |
| { Milieu   | 9 72               | 11 76 | 11 11                  | 69 11 | 13 88                   | 107 12 |                          |   |
| { Fin  | 0                  | 2 91  | 19 44                  | 73 52 | 29 16                   | 136 74 |                          |   |
| (dihydroamino-pentyl) amino-4 chloro-7 quinoléine (Nivaquine B)+Rodopréquine (Prémaline N) |                    |       |                        |       |                         |        |                          |   |
| <i>Dar Caid Ahmed II</i>   |                    |       |                        |       |                         |        |                          |   |
| (91 adultes+72 enfants)  |                    |       |                        |       |                         |        |                          |   |
| { Début  | 12 08              | 22 22 | 17 58                  | 77 77 | 21 97                   | 144 08 | 4                        | 8 |
| { Milieu   | 9 89               | 41 66 | 10 94                  | 79 10 | 15 37                   | 140 14 |                          | 2 |
| { Fin  | 0                  | 9 72  | 8 79                   | 66 66 | 12 04                   | 125 95 |                          |   |
| Quinacrine+Rodopréquine (Prémaline)  |                    |       |                        |       |                         |        |                          |   |
| 4 <sup>ème</sup> GROUPE  |                    |       |                        |       |                         |        |                          |   |
| <i>Hadada</i>  |                    |       |                        |       |                         |        |                          |   |
| (40 adultes+20 enfants)  |                    |       |                        |       |                         |        |                          |   |
| Témoin { Début   | 15                 | 13 79 | 32 5                   | 89 63 | 43 55                   | 164 34 |                          | 4 |
| { Milieu   | 17 4               | 17 24 | 27 5                   | 75 86 | 36 02                   | 177 41 |                          |   |
| { Fin  | 7 5                | 31 03 | 27 5                   | 89 63 | 55                      | 196 33 | 1                        |   |
| <i>Dar Fedell I</i>  |                    |       |                        |       |                         |        |                          |   |
| (51 adultes+52 enfants)  |                    |       |                        |       |                         |        |                          |   |
| { Début  | 0                  | 13 46 | 33 33                  | 88 48 | 40 99                   | 141 52 |                          | 1 |
| { Milieu   | 3 92               | 23 07 | 11 71                  | 73 07 | 11 71                   | 119 10 |                          |   |
| { Fin  | 3 92               | 9 41  | 7 84                   | 50 61 | 9 60                    | 95 97  |                          |   |
| N <sup>1</sup> -p-chlorophényl N <sup>1</sup> isopropyl bisulfamide (J 359 R 1)            |                    |       |                        |       |                         |        |                          |   |
| <i>Dar Fedell II</i>   |                    |       |                        |       |                         |        |                          |   |
| (54 adultes+56 enfants)  |                    |       |                        |       |                         |        |                          |   |
| { Début  | 10 71              | 15 78 | 21 92                  | 83 71 | 29 54                   | 159 47 |                          |   |
| { Milieu   | 8 57               | 25 07 | 9 25                   | 73 21 | 9 25                    | 147 88 |                          |   |
| { Fin  | 3 70               | 12 50 | 3 54                   | 75    | 7 33                    | 138 8  |                          |   |
| Quinacrine+Rodopréquine (Prémaline)  |                    |       |                        |       |                         |        |                          |   |

\* Pour les index épidémiologiques les termes début milieu et fin de l'expérience correspondent aux dates indiquées plus haut

Ces résultats ne sont pas exempts de critiques car, du fait du jeûne diurne du Ramadan, la prophylaxie fut suspendue pendant la période de un mois précédant l'établissement des index de la m<sup>1</sup> prophylaxie, ce qui explique leur élévation

D'autre part, un certain nombre d'adultes mangèrent plusieurs distributions

Néanmoins, les résultats sont comparables entre eux

TABLEAU 2

|   | Index plasmodiques |       | Index splé-<br>niques |       | Index spléni-<br>métriques |        | Accès<br>palustres<br>confirmés |   |
|---|--------------------|-------|-----------------------|-------|----------------------------|--------|---------------------------------|---|
|   | A                  | E     | A                     | E     | A                          | E      | A                               | E |
| <b>1<sup>er</sup> GROUPE</b>  |                    |       |                       |       |                            |        |                                 |   |
| <i>O B Tabala</i>   |                    |       |                       |       |                            |        |                                 |   |
| (109 adultes + 96 enfants)  |                    |       |                       |       |                            |        |                                 |   |
| Témoin { Début :  | 4 62               | 23    | 13 76                 | 47 81 | 30 58                      | 86 23  |                                 | 1 |
| Milieu  | 8 33               | 43 75 | 18 34                 | 70 83 | 33 37                      | 140 24 |                                 |   |
| Fin   | 4 62               | 35 41 | 23 85                 | 71 87 | 64 63                      | 173 20 | 1                               | 5 |
| <i>Dekakis</i>  |                    |       |                       |       |                            |        |                                 |   |
| (115 adultes + 91 enfants)  |                    |       |                       |       |                            |        |                                 |   |
| { Début   | 2 46               | 27 47 | 12 17                 | 40 65 | 19 06                      | 43 08  |                                 |   |
| Milieu  | 10 25              | 80 76 | 11 39                 | 43 95 | 27 79                      | 86 36  |                                 |   |
| Fin   | 1 70               | 8 49  | 6 69                  | 39 56 | 27 34                      | 48 29  |                                 |   |
| (diéthylaminopentyl) amino-4 chloro-7<br>quinoléine (N) (vaquine + Rodopréquine<br>(Primaline N)) |                    |       |                       |       |                            |        |                                 |   |
| <i>Risk Ouélla</i>  |                    |       |                       |       |                            |        |                                 |   |
| (110 adultes + 94 enfants)  |                    |       |                       |       |                            |        |                                 |   |
| { Début   | 9 73               | 27 45 | 10                    | 48 80 | 22 70                      | 115 19 |                                 |   |
| Milieu  | 15 27              | 55 31 | 17 27                 | 50    | 30 74                      | 95 50  |                                 |   |
| Fin   | 6 30               | 8 81  | 14 54                 | 22 67 | 27 95                      | 89 34  |                                 |   |
| N-p-chlorophényl N-isopropyl biguan<br>ide (2 359 R P) + Rodopréquine                             |                    |       |                       |       |                            |        |                                 |   |
| <b>2<sup>ème</sup> GROUPE</b>   |                    |       |                       |       |                            |        |                                 |   |
| <i>Ouéd Aris</i>  |                    |       |                       |       |                            |        |                                 |   |
| (108 enfants)   |                    |       |                       |       |                            |        |                                 |   |
| Témoin { Début  | 27 77              |       | 34 23                 |       | 70 21                      |        | 7                               |   |
| Milieu  | 40 74              |       | 48 14                 |       | 84 72                      |        | 4                               |   |
| Fin   | 23 77              |       | 60                    |       | 176                        |        | 19                              |   |
| <i>Ouéd Ziène I</i>   |                    |       |                       |       |                            |        |                                 |   |
| (66 enfants)  |                    |       |                       |       |                            |        |                                 |   |
| { Début   | 18 69              |       | 25 73                 |       | 81 67                      |        | 2                               |   |
| Milieu  | 63 33              |       | 33 33                 |       | 81 99                      |        | 4                               |   |
| Fin   | 18 66              |       | 24 34                 |       | 31 02                      |        | 2                               |   |
| Quinacrine + Rodopréquine (Primaline)   |                    |       |                       |       |                            |        |                                 |   |
| <i>Ouéd Ziène II</i>  |                    |       |                       |       |                            |        |                                 |   |
| (70 enfants)  |                    |       |                       |       |                            |        |                                 |   |
| { Début   | 24 28              |       | 21 42                 |       | 33 41                      |        |                                 |   |
| Milieu  | 32 75              |       | 25 71                 |       | 34 96                      |        | 1                               |   |
| Fin   | 5 71               |       | 18 67                 |       | 26 34                      |        | 1                               |   |
| N-p-chlorophényl N-isopropyl biguan<br>ide (2 359 R P)  |                    |       |                       |       |                            |        |                                 |   |
| <b>3<sup>ème</sup> GROUPE</b>   |                    |       |                       |       |                            |        |                                 |   |
| <i>Semara</i>   |                    |       |                       |       |                            |        |                                 |   |
| (20 adultes + 15 enfants)   |                    |       |                       |       |                            |        |                                 |   |
| { Début   | 20                 | 13 33 | 23                    | 80    | 35                         | 120    | 9                               | 3 |
| Milieu  | 0                  | 23 33 | 50                    | 80    | 80                         | 152 3  |                                 |   |
| Fin   | 0                  | 26 66 | 23                    | 60    | 30                         | 160    |                                 |   |

See footnote at end of table.

(2°) Protection d'une population européenne indemne de paludisme, amenée dans une région d'endémie palustre (Oued el Lal, Tunisie)

deux des accès à *P. vivax*

Les témoins de cette expérience furent les 800 ouvriers indigènes de l'entreprise où, pendant cette période, on observa 319 cas de paludisme confirmé

Ainsi, en prophylaxie individuelle, la Nivaquine, à la dose de 0g 10 par jour est bien tolérée et confère une protection absolue

### CONCLUSIONS

(1°) le chlorhydrate de méthyl 3 (diéthylaminopentyl) amino-4 chloro-7 quinoléine (Sontoquine C—Nivaquine C—), le sulfate et le diphosphate de (diéthylaminopentyl) amino-4 chloro 7 quinoléine (Résoquine, N—Paludrine) avec une égale activité sur les schizontes de *P. falciparum*, *vivax* et *malariae*

(2°) Nos premières expériences de prophylaxie collective démontrent qu'une dose hebdomadaire de 0g 30 de N<sup>1</sup>.p chlorophényl N<sup>1</sup>.

conclusion définitive.

à la Commission détaillée Les

L  
7/1948.

### ABSTRACT OF DISCUSSION

Johnson). During the  
sup  
was  
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(2<sup>e</sup>) EXPERIENCE DE TUNISIE

Cette expérience fut faite dans des conditions analogues mais ici, on put faire une distribution nocturne de médicaments pendant le Ramadan

Les résultats sont rapportés dans le tableau 3

TABLEAU 3

| Numéro de secteur | Traitement               | Nombre de sujets | Index spléniques globaux |        |            | Index plasmodiques globaux |        |            | Cas de paludisme confirmés |
|-------------------|--------------------------|------------------|--------------------------|--------|------------|----------------------------|--------|------------|----------------------------|
|                   |                          |                  | 1 Mai                    | 2 Août | 3 Novembre | 1 Mai                      | 2 Août | 3 Novembre |                            |
| A                 | 3500 R P + Rodop         | 106              | 15.05                    | 17.97  | 8.06       | 10.21                      | 2.64   | 2.13       | 4=2.04%                    |
| B                 | 3500 R P                 | 205              | 14.11                    | 6.73   | 6.69       | 8.29                       | 1.03   | 2.64       | 1=0.48%                    |
| C                 | Témoins                  | 100              | 12.48                    | 16.66  | 15.29      | 6.73                       | 17.45  | 8.21       | 21=21%                     |
| D                 | Prémaline                | 465              | 9.50                     | 13.11  | 6.26       | 10.25                      | 1.61   | 1.35       | 2=0.43%                    |
| E                 | Témoins                  | 160              | 16.17                    | 25.74  | 23.71      | 11.8                       | 13.20  | 8.97       | 22=13.75%                  |
| F                 | Niva B+Rod (Préma Ene N) | 152              | 12.5                     | 11.87  | 10.53      | 9.37                       | 3.12   | 62         | 2=1.00%                    |

En conclusion, cette série d'expériences de prophylaxie montre que les nouveaux antipaludiques sont au moins aussi actifs que la Prémaline et ils ont l'avantage de ne pas être colorés, mais on ne peut encore tirer de conclusions définitives sur leur supériorité respective, la première impression est que l'association Nivaquine + Rodoprequine est légèrement plus active que les autres

Nous avons montré que dans le traitement curatif, la Paludrine a une action plus lente sur *P. vivax* et sur *P. malarie* que sur *P. falciparum*. En prophylaxie ou le traitement dure longtemps, cet inconvénient disparaît car, après plusieurs semaines, l'action est la même sur les différentes plasmodies

Actuellement, des expériences de prophylaxie sont en cours utilisant des doses et des rythmes variables de distribution, elles nous permettront d'apporter des conclusions sur l'action respective des nouveaux antipaludiques.

B, Prophylaxie individuelle. Ces expériences ont porté, jusqu'à présent, sur la Nivaquine selon deux types

(1<sup>re</sup>) Prophylaxie d'une fraction de population indigène déjà impaludée vivant au milieu d'une population non soumise à la prophylaxie (Ghardimaou, Tunisie)

L'expérience a porté sur 106 individus prenant individuellement 0g 10 tous les jours pendant 6 mois.

Ce traitement bien toléré, abaissa l'index splénique de 10,4 pour cent à 4,73 pour cent et l'index plasmodique de 4,73 pour cent à 0 pour cent, alors que chez les 640 témoins, l'index plasmodique passait de 3 à 10 pour cent

Il ne fut observé aucun accès de paludisme parmi les 106 personnes traitées, alors que, dans le même temps, on notait 160 cas de paludisme falciparum confirmé chez les 640 témoins

still very high and some adult forms had appeared in the peripheral circulation which, with our *falciparum* strain, is considered a premonitory sign of a malignant attack. In fact, the patient's condition became very serious, vomiting appeared, so that we were obliged to give him two injections of gram 10 of quinine within about 2 hours. The quinine treatment was continued.

Another patient, with a malignant tertian infection, was not a primary case, he was treated only with paludrine, and fever and the parasites disappeared in a few days.

We experimented also with paludrine and palusil on chickens infected with *P. gallinaceum*. According to the weight of the chickens treated we gave, as a rule, a dose which in man would correspond to about gram 10 per day. Parasites disappeared in from 3 to 7 days. After the second day of treatment, only gametocytes were found in the blood of the treated chickens. Treatment lasted 7 days in seven chickens and 14 days in one chicken.

The blood of five infected chickens, inoculated at the end of the treatment into healthy chickens always proved to be infective. One of the infected chickens was given two successive treatments and yet its blood proved to be infective after both the treatments.

In the smears from liver cuts of one of these chickens it has not been possible up to now to detect any erythrocytic forms.

Dr G. ROBERT COATNEY (United States). We, at the National Institute of Health, have tested most of the drugs discussed here this morning against *Plasmodium vivax* under controlled conditions in

prisoner volunteers  
paludrine  
cellent series

11

to quinine and quina  
is the drug of choice?

Naturally, neither one has withstood sufficient field trials for definitive answers. In our experience, however, both are excellent suppressants of the Chesson strain (Southwest Pacific) of *vivax* malaria in dosage of 0.3 gram once weekly, but even after a year of such suppress-

t dose of  
margin  
various  
t of the

drug for field use. I wonder if Professor MacGillivray has any information on this point?

Dr LEONIDAS M. DEANE. Since the new synthetic drug, *ca* hydrochloride, has not been brought into this discussion, we should like to mention that, in Brazil, this drug has proved quite promising

doses were used for children. We found that when five tablets were used, a single course of treatment cured clinically 75 percent of cases, but when 2.5 gram doses (10 tablets) were used, all cases made a speedy clinical recovery by a single course of treatment.

To build up a high-plasma level in the shortest time possible, 2 tablets (0.5 gram) were given at once, 2 tablets 1 hour and 2 more

and none of our patients had a second paroxysm. The parasites

relapsed (12.5 percent). These were from among those who received

quine, and paludrine in the following respects: (1) clinical response to therapy was more rapid, (2) destroyed plasmodia in peripheral blood in a shorter time; (3) the standard course of treatment (3

called palusil which has the same chemical composition as paludrine.

In the human malaria, we administered to the patients the doses suggested by the producing firm, that is, gram 0.30 of paludrine for 10 days and gram 0.20 for 14 days.

In the treatment of *P. vivax* infections, results have been satisfactory; clinical symptoms and parasites disappeared in a period from 3 to 5 days.

In the treatment of *P. falciparum* infection, results were not so good. One of the treated patients was suffering from a very serious infection, with tenderness

0.20  
the s



Dr J RODUAIN (Belgium) J'ai suivi avec le plus vif intérêt les différentes lectures présentes au cours de cette session. Elles nous ont montré les plus récentes acquisitions dans le domaine de la chimiothérapie antimalarienne. J'ai la plus profonde admiration pour les chercheurs qui par leurs efforts laborieux ont enrichi notre arsenal antipaludique. Je sais les énormes services que l'atèbrine a rendu durant la guerre mondiale et je suis convaincu de ce que les nouveaux produits synthétisés ont une valeur curative et prophylactique considérable. Mais je suis un vieux clinicien et c'est à ce titre que je desire insister sur le fait que l'action toxique des nouveaux médicaments n'a pas été suffisamment considérée dans le temps.

Tous ces médicaments sont jeunes. Leur toxicité que j'appellerai présente ou immédiate a été étudiée avec beaucoup de soin sans doute mais leur toxicité tardive ne peut encore être connue.

En disant cela, je pense à ce qui s'est produit pour la plasmochine et l'atèbrine.

J'ai vu apparaître la plasmochine on la portait aux nues, recommandant 8 centigrammes par jour. Mais bientôt cette dose dut être réduite à 5 puis à 3 centigrammes. Je puis assurer ici que certains malades ne supportent pas sans troubles cardiaques et circulatoires journaliers les 3 centigrammes journaliers.

des troubles psychiques peut être réelle. Que se produira-t-il si la durée d'emploi s'étend à 20 ans ou plus chez le même sujet.

Et la remarque que je veux faire, est que ce ne sera que lorsque les nouveaux synthétiques auront subi l'épreuve du temps que nous serons fixés sur leur innocuité réelle, leur activité thérapeutique n'étant d'ailleurs aucunement mise en doute.

Dr M NIETO CAICEDO (Venezuela) Como una contribución a las exposiciones efectuadas, vamos a exponer los resultados obtenidos en Venezuela durante dos años de experiencias con cloroquina, llevadas a cabo en los servicios de la División de Malaria, en la cual prestamos nuestros servicios. Los objetivos perseguidos en las experiencias efectuadas, fueron:

(a) Determinar si era posible el control de la transmisión malarica por medio de la terapéutica supresiva con cloroquina a dosis supresivas semanales.

(b) Determinar si era posible el control de la morbilidad y de la mortalidad por malaria, con cloroquina a dosis supresivas semanales de 0.30 grs. de la base para el adulto.

(c) Saber si la cloroquina ofrecía ventajas sobre los anteriores tipos de tratamientos empleados en Venezuela de metoquina y quinina, dis-

# V MALARIA

in the hands of Mem, Rosado, and coworkers as reported them last October, at the 6th Brazilian Congress of Hygiene. Dr Victor A. Sutter and myself are now conducting an experiment a small Amazonian village, in order to determine if camoquin hydrochloride can be used in that region as a reliable malaria suppressant. Camoquin hydrochloride was given to the entire population of Acar (about 200) which was then divided into two groups, one of which receives weekly a suppressive dose of camoquin hydrochloride, while the other group, the control group, takes saccharose tablets. During the weekly examination of the inhabitants, blood slides are made of all who have had fever. Those with malaria symptoms, both in the suppressive group as well as in the control group, are given curative doses.

Ten weeks from the beginning of the experiment, there were already 10 slides with plasmodia in the control group and none in the group which is undergoing suppressive treatment.

In the course of this experiment and the examination of a few malaria cases in Belém, Dr Sutter and I were able to give 1 single dose of camoquin hydrochloride to 133 malaria patients with positive blood slides. Examination of thick and thin smears 7 days afterward revealed that 27 cases (20 percent) still showed plasmodia, but the only resistant forms were the gametocytes of *falciparum*. Of 88 malar cases (among which there were 77 with gametocytes) all were negative 1 week after the administration of 1 single dose of camoquin hydrochloride, of 42 *falciparum* infections of which there were 34 with gametocytes 27 remained positive, all with gametocytes only. Two infections of *malariae* (with schizonts and gametocytes) were also treated with a single dose and both were negative 1 week after wards.

In 32 patients whose blood was collected daily after treatment, until fever was negative for three consecutive days it has been seen that of 26 malar cases 7 became negative on the first day, 17 on the second day and 2 on the third day. *Falciparum* gametocytes, however, could be found in the peripheral blood many days after the curative dose one case which was followed daily up to 21 days after treatment, still positive for those forms. In the 2 malar cases treated, parasites persisted in the peripheral blood for 3 and 4 days, respectively, after the administration of the drug. These are only preliminary results of an experiment that is being carried on.

Curative scheme used, as to dosage is the following

From 0 to 24 months 0.1 gr base  
 from 1 to 4 years 0.2 gr base  
 from 5 to 9 years 0.3 gr base  
 from 10 to 14 years 0.4 gr base  
 from 15 years up 0.5 gr base

## Suppressive

From 1 to 4 years 0.1 gr weekly  
 From 4 to 14 years 0.2 gr weekly  
 From 15 years up 0.3 gr weekly

morbosidad, la cual permanece dentro de valores bajos, en los meses siguientes a la terminación de aquella.

61 La inmunidad específica de la población, valorada por el índice esplénico escolar y la esplenomegalia media, experimenta descenso consecutivamente a la terapéutica supresiva.

II Conclusiones en las experiencias comparativas, con la quinina y la metoquinina

1a La cloroquina se muestra en relación con los tipos de tratamientos rurales utilizados en Venezuela y ante cepas venezolanas de plasmodios notablemente más activa que la quinacrina y, la quinina al dominar la parasitemia y la fiebre, en las infecciones por *P. vivax* y *P. falciparum*, en tiempos muchos más cortos.

2a Dosis totales de cloroquina base, que oscila entre 0.90 a 1.80 gramos, administradas en 1, 2, 3 o 4 días, muestran todas, buenos efectos curativos del ataque agudo.

3a En las infecciones por *P. falciparum* se observa una mayor resistencia del parásito ante la droga, por lo que se considera conveniente que el tratamiento tenga una duración no menor de 3 a 4 días para asegurar una alta concentración plasmática del medicamento, mientras existan formas asexuadas en la sangre circulante.

4a El tratamiento de un solo día de duración, se considera útil para las infecciones por *P. vivax*, especialmente cuando interese la administración personal de la droga.

5a La cloroquina muestra acción gametocida en las infecciones por *P. vivax* y *P. malariae*. En las infecciones por *P. falciparum* no inhibe la formación de gametocitos ni destruye los ya formados.

6a Los trastornos que se han observado durante la administración de la cloroquina y que pudieran ser ocasionados por esta droga excepto en dos casos, carecieron de importancia. Todos los síndromes tóxicos sin excepción, evolucionaron favorablemente, en corto plazo. Un síndrome psicótico observado se considera relacionada con la existencia de una tara hereditaria.

7a La cloroquina reduce, en los enfermos de paludismo, el número de estancias hospitalarias, en 5 días, en relación con el tratamiento con sales de quinina y, en 3 días, en relación con el tratamiento con quinacrina.

III Conclusiones sobre la aplicación en terapéutica infantil

1a La cloroquina puede ser administrada a niños, a la dosis diaria  
cual dosis son  
odos los ataques  
; como máximo

todos los producidos por *P. falciparum*, el tiempo medio de desaparición de los trofozoitos y esquizontes en las infecciones por *P. vivax* es de 1.58 días y en las infecciones por *P. falciparum* de 2.14 días. La temperatura se normaliza en 1.4 días en los primeros y en 2.42 en los segundos.

2a Una dosis semanal hasta de 0.15 gramos, puede ser administrada

tribuidos colectivamente para el tratamiento de los ataques agudos de malaria

(d) Determinar, si era posible emplear la cloroquina, en niños menores de 5 años a dosis supresivas y curativas

Las experiencias fueron efectuadas en el pueblo de Santa Apolonia, de 700 habitantes, situado en el Estado Trujillo, región del Lago de Maracaibo. El vector allí es *Anopheles darlingi*, el índice parasitario global antes de la experiencia fue de 41% y el índice esplenico escolar fue de 100%. Es pues una localidad de alta endemia malarica. El personal empleado fue sometido a constante supervisión.

Presentamos a continuación los resultados obtenidos

I Conclusiones en las experiencias con terapéutica supresiva

1a El control quimioterápico de la transmisión malarica actuando sobre el reservorio del virus, se considera perfectamente factible con la administración semanal de cloroquina a la dosis de 0.30 grs para el adulto, siempre que se administre esta a toda la población. En el pueblo de Santa Apolonia en Venezuela, durante el año de la experiencia persistió la transmisión solo en el núcleo de población infantil no tratada (menores de 5 años) el cual fue utilizado como grupo de control, para el cual no existían pautas de tratamiento conocidas. El grupo tratado con cloroquina en las 4 semanas de cada mes, presentó índices de infección y gametocíticos, insignificantes en las personas

supresivos se mantienen aptos para el trabajo y con sensación subjetiva

puede mantener bajo control con terapéutica semanal supresiva y

tomado muestras. Se estima que en casos de epidemias, el dejar la terapéutica supresiva en manos de los propios habitantes, no per-

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we have treated 89 cases, they were primary or relapsing with this new drug. The oldest 72 years old and after treatment, every 24 hours. Temperature, pulse, and respiratory rates were taken every 4 hours. Our cases are divided into the following categories:

*Plasmodium vivax*, 64 cases, *P. falciparum*, 21 cases, *P. malariae*, 2 cases, mixed, 2 cases. A half gram of camoquin was given. This is a single dose treatment. We just gave the drug one time to these patients.

With this dosage we have observed the following:

The control of temperature, within 30 hours, whether it be a *falciparum*, *vivax* or malarial infection. In the peripheral blood picture as to *Plasmodium vivax*, within 48 hours all blood smears are negative.

and 72 hours

and we have had no untoward symptoms.

As to the relapse rate, it is very difficult to control, or to know whether you are dealing with a relapse case or primary infection in the highly endemic area. But the cases of the hospital personnel, nurses, orderlies, chauffeurs, we have been able to control. We have some cases where the blood smears have continued negative after 18 months of treatment.

Dr M. PLOY (France) I am of the opinion (a) that the optimal

quinine  
it would

For all these reasons, I recommend the use of a universal standard quinine in the form of tablets of 20 centigrams of quinine hydrochloride. Such a standardized presentation would not only make for a more precise posology but, in reducing the cost of production, would allow a better regulated and wider mass treatment.

Dr AHMED HALAWANI (Egypt) I would just like to say a few words regarding this camoquin. Camoquin has been tried in Egypt in 1946 and early 1947. The drug can be given in two doses in one day,

# V MALARIA

a niños de 1 a 4 años, durante meses con buenos efectos supresivos que se observen manifestaciones tóxicas.

3a La acción sobre los gametocitos de *P. vivax* es rápida y completa sobre los gametocitos de *P. falciparum* muestra igual ineficacia que ya observada entre los adultos.

Dr. PIR DECOTER (France) Chez l'homme la pentaquine est effectivement moins toxique que la pamaquine, mais elle est aussi moins active de sorte que l'index thérapeutique reste le même.

La rhodoquine ou 710 Fourneau a la même activité que la pamaquine, mais sa toxicité est très différente, elle ne produit pas de troubles sanguins. Des expériences de laboratoire, confirmées depuis par de multiples applications cliniques, ont permis de démontrer qu'en associant pamaquine et rhodoquine, les activités s'additionnent alors que les toxicités restent dissociées. Il en résulte que cette association, appelée rodopraquine, a une toxicité correspondant a une dose moitié moindre de pamaquine et celle de la pentaquine. Inversement la rodopraquine est environ deux fois plus active que la pentaquine, son activité étant égale à celle de 6 centigrammes de pentaquine, son

Il n'est pas 3 centigrammes de rodopraquine ont une activité sensible égale à celle de 15 centigrammes de pamaquine, et la toxicité de la rodopraquine a été largement utilisée dans les expériences cliniques françaises en association avec la quinacrine. En prophylaxie 30 000 personnes traitées simultanément 5 mois par an pendant 9 ans et pendant 2 autres années à 80 000 personnes sans qu'aucun effet toxique ait été constaté. Elle a été très largement utilisée de la même façon dans d'autres territoires français.

Au cours des dernières années la rodopraquine a été aussi utilisée sur plusieurs milliers de personnes en association avec la nivaquine ou chloroquine et avec la paludrine sans incident.

En ce qui concerne le traitement par l'association de pentaquine et de nivaquine pendant 14 jours il est difficile d'appliquer un traitement aussi régulier thérapeutique habituelle. Il serait en tout cas impossible d'appliquer normalement dans les populations indigènes que nous traitons les territoires français. Nous devons, au contraire utiliser, pour les populations des traitements aussi courts que possible et ne dépassant 4 à 5 jours. A ce point de vue, comme au point de vue prophylactique nous utilisons de préférence la Primatine constituée par l'association de 10 parties de Nivaquine et une partie de Rodo-

ROBERT W. MEN (Brazil) I would like to speak about our trials with a new drug cinmoquin. It is a quinoline derivative manufactured by Parke, Davis & Co.

have been working in Belém, Para, which is a highly endemic area for malaria. All of these cases were hospital cases. To date

## Session 4 IMMUNITY, MALARIA CONTROL

Friday, May 14—2 to 4 30 p m

Departmental Auditorium, Main Hall

### ACQUIRED IMMUNITY IN MALARIA

WILLIAM H TALIAFERRO, *Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill*

Although our chief interest lies in acquired immunity, any consideration of acquired immunity should be prefaced by recalling the probably universal occurrence and efficacy of innate immunity. From the very beginning of the malarial infection and extending, in general, through the acute rise, as recognized by Gelgi in 1888 and as studied statistically in detail by modern workers, especially L. G. Taliaferro (1925) and Hartman (1927), up to 66 percent of the progeny produced at each segmentation may perish (see review in Taliaferro and Mulligan, 1937, and Taliaferro, 1948b). The actual number that die depends upon various features of the parasite and host. In addition, certain races of man and even individuals of the same race possess, on the average, more innate immunity than others. Acquired immunity, if developed, is superimposed upon this base of innate immunity.

As time does not permit a systematic review, I shall limit my remarks to a few characteristics of acquired immunity with special reference to some of the more recent work and some of the unsolved problems in the field.

The test for the presence of acquired immunity is almost exclusively based upon some modification in the parasitemia, such as the suppression of initial or superinfections to varying degrees. We greatly need some in vitro test for functional acquired immunity. The protection test has been perfected for only a few infections and in mon-

volves strain specific antigens. The agglutination reaction, on the other hand, as first described by Eaton (1938) for *Plasmodium knowlesi*, shows promise because the phenomenon occurs in vivo in some malarias as part of the immune reaction and, as far as is known, is strain specific. The opsonic test, as developed recently by Zucker-  
man (1945) with macrophages grown in tissue culture, is also prom-

or in one dose. The results obtained are excellent, satisfactory compared to other drugs used in malaria. But it has had some effects and patients complained of dizziness and headache more often than with other drugs, such as paludrine.

I would like to mention one observation which has been made, that is that nivaquine, camoquine and paludrine have been used in treatment of divers cases. Ninety three cases received nivaquine, camoquine, and 99 paludrine. These were observed over the period between the end of the season of malaria and the beginning of the second season. Out of the 99 cases treated with paludrine, 1 case relapsed. Out of the 83 cases receiving camoquine, 4 cases relapsed while out of the 93 cases treated with nivaquine, 10 cases relapsed.

Dr A T KROONERS (Netherlands). I want to pay tribute to the important work of Dr Alving and his collaborators in this field. The antirelapse activity of quinine and pamaquine was always of great interest in Holland. Already in 1932, immediately after the discovery by Sinton the value of this therapy was confirmed in Holland and Irisin. A description of the experiments appeared in the book, *Malaria in the Netherlands* by Swellengrebel and de Buck. We now treat the vivax malaria in North Holland with 900 milligrams quinine sulfate and 54 milligrams pamaquine naphthate a day during 14 days, we get a very low relapse rate, but we hope to obtain more thorough drugs than pamaquine. In my opinion a lot of strains will react readily on this low dosage but it must be estimated strain for strain in every country. In North Holland hospitalization is not necessary, the patient is warned and can get medical care if he wants it. This dosage scheme very rarely exhibits toxic symptoms.

In experiments on gallinaceum malaria, we found a fact that may help us in the explanation of the synergism of quinine and pamaquine on the erythrocytic forms. We succeeded in obtaining a twofold quinine resistance in *P. gallinaceum*, with the chicken as host by giving for weeks a dosage which caused a 50 percent reduction of the infected erythrocytes in the technique of Davey (every week blood inoculation and s f). After 26 weeks, a twofold resistance was clearly demonstrable. This resistant strain is normally sensitive to atabrin, paludrine, chloroquine and sulphonamides. It is however, more sensitive to pamaquine and pentaquine as compared with the normal control strain. In experiments on the explanation of the synergism of quinine and pamaquine on the erythrocytic forms, one could venture the hypothesis that the more resistant part of the strain to quinine is hypersensitive to 8 aminoquinolines. This, however only explains the synergism in the erythrocytic forms. The meeting adjourned at 12.45 p.m.)



by repeated superinfections with *P. lophurae* and *P. gallinaceum* but not in chickens recovering from initial infections. The antibodies in her work behaved like immune iso hemopsonins and may have resulted from her use of blood transfusions from one chicken to another during the procedure of hyperimmunization.

In view of the foregoing positive results, there is, as yet, no satisfactory explanation for the difficulty in demonstrating protective antibodies in such infections as *P. cathemerium* in which intense immune reactions occur. Several suggestions have been made, all of which may partly account for the difficulty.

Cannon and I (1936) have suggested that antibodies are formed locally in the sites of macrophage activity in sufficient concentrations to be effective locally but not in sufficient concentrations to be readily transferred passively after dilution in the blood stream.

On occur the re meability of the surrounding red cell or unless it is free in the plasma. Nevertheless, he stresses the need for using small doses of organisms in protection tests.

Mulligan and his coworkers (1940) believe that antibodies are not fully effective unless the lymphoid macrophage system is sufficiently activated. This activation was brought about in their work on *P. knowlesi* by a previous infection with the antigenically distinct *P. cynomolgi*.

Others have stressed the probably significant role of nonimmunological factors. Thus, recently Rigdon and his coworkers (Rigdon and McCain, 1947), in studying the mechanism of the parasite decline in infections with *P. lophurae*, believe that many parasites are killed

factors, it should be kept in mind that the high degrees of immunity to superinfection persist during long periods of latency when many, if

among different stages of the parasite. M. T. Boyd and Kitchen (1936) and Sinton (1940) believe acquired immunity to *P. vivax* and *P. ovale* in man, whether following blood induced or sporozoite induced infection, is largely directed against the erythrocytic stage rather than the sporozoite. Russell, Mulligan, and Mohan (1942)

th inactivated sporozoites to sporozoite but not to ton (1946) have recently demonstrated that apparently normal preerythrocytic stages but few or no blood stages of *P. gallinaceum* develop when large numbers of

ising. Theoretically, this test most nearly approaches the situation *in vivo* but is technically difficult and has not been used in the primar-  
malarial

The primary role of the macrophages of the spleen, liver, and bone marrow during both innate and acquired immunity in phagocytosis of free parasites and parasitized erythrocytes has been demonstrated by many investigators beginning with Laveran, Golgi, Metchnikoff and Marchafava and Celli between 1884 and 1888, and especially by Cannon and me (1931 and 1936), who made closely spaced observations on avian and simian infections. In addition, macrophages increase according to work by Mulligan and me (1937) is due only in very small part to the homoplastic development of macrophages, the mitotic division of reticulo endothelial hyperplasia. It is predominantly due to the heteroplastic development of macrophages from lymphocytes (with or without an intermediate monocyte stage). Thus, the lymphocyte forms a mesenchymal reserve from which macrophages can arise in malaria, as has been shown to take place in local inflammation by Vivumow, Bloom and others.

The role of the lymphocyte in malarial immunity is further indicated by my work done in collaboration with L. G. Taliaferro and Simmons (1915 and 1916). We found that such lymphocytocidal agents as large doses of A rays applied to the entire animal or of one of the nitrogen mustards given intravenously sometimes markedly reduce the immunity of chickens to *P. lophurae* and *P. gallinaceum*. The extent of the reduction depends upon the virulence of the parasite and the degree of immunity present. These agents destroy lymphocytes and probably interfere with the very necessary heteroplastic development of macrophages from lymphocytes. They may also interfere with the formation of antibodies by lymphocytes in malaria the lymphocyte is involved in antibody formation as indicated (1) the work of Hektorn and others on the effect of lymphocytocidal agents in reducing antibody formation and (2) by recent investigations in which antibodies have been found within lymphocytes. The most striking histological evidence of acquired immunity which mon and I (1936) found is the greatly increased phagocytosis of parasites and parasitized erythrocytes. This increase we and others found to be due to an opsonin. Evidence for the occurrence of such failures or inconclusive results by previous investigators Coggeshall and Kumm (1937) after monkeys after the onset of acquired immunity during an initial infection or after repeated superinfection with *P. knowlesi* or *P. vivax* findings have subsequently been corroborated and reported for malarial. In addition, Zuckerman (1915), in her *in vitro* found an opsonin in serum from chickens hyperimmunized

(1939), Coggeshall (1913) and others, have implications in regard to immunization and the supplementary role of acquired immunity in treatment. In the first group of infections, antigenic stimulation may be reduced in early treatment to such a point that acquired immunity plays only a minor supplementary role in chemotherapy, whereas in the second group, antigenic stimulation, even though reduced by early treatment, is strong enough to evoke acquired immunity. Similarly much larger amounts of vaccine may be necessary in the first group than in the second one to produce similar degrees of immunity.

The tendency of malaria to relapse makes the use of living vaccines dangerous. Effective noninfectious vaccines for artificial immunization have only recently been prepared. With erythrocytic stages Gingrich (1941) first obtained partial immunity by injecting canaries with large numbers of heat or formalin killed *P. cathemerium*. Jacobs (1943) increased the efficacy of *P. lophurae* vaccines by adding *Staphylococcus* toxoid. More recently, Freund and his coworkers (1945) and Thomson et al., (1947) have reported high degrees of acquired immunity against *P. lophurae* in ducks and against *P. knowlesi* in monkeys after immunization with formalin killed parasites mixed with paraffin oil containing killed tubercle bacilli and an emulsifying agent. These are the most promising results so far obtained.

Working with *P. gallinaceum*, Mulligan, Russell, and Mohan (1941) were the first to obtain partial immunity in chickens with a vaccine produced by inactivating sporozoites of *P. gallinaceum* with ultra violet light. Russell and Mohan (1942) further found that protection was increased when vaccination was combined with the passive transfer of immune serum.

So far, however, attempts to protect human malaria have been disappointing. Large numbers of blood parasites are necessary (1) to influence the course of the infection with *P. vivax* or (2) to obtain appreciable protection against blood induced infections with the McCoy strain or against sporozoite induced infections with the Chesson strain of *P. vivax*.

There is little hope at present that potent antiserums or dead vaccines will be developed and widely used to control human malaria. In either passive or active immunization, the number of antigenic variants included in each species is a great, if not insurmountable

barrier. The present outlook is gloomy. If success is attained

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sporozoites are injected intradermally into chickens immunized blood induced infection or by repeated reinoculations of sporozoites. To explain such differences the authors have suggested that different antibodies act against the sporozoite and against the erythrocytic stages.

There seems little disagreement today that acquired immunity is an important aid in the drug treatment of malaria. Treatment is easier in individuals with an appreciable immunity and is almost certainly supplemented by acquired immunity in those cases in which cessation of suppressive drugs is not followed by early relapses. Recently (1947, 1949), with L. G. Taliaferro and Kelsey, I have systematically studied the role of the spleen in the antimalarial activity of quinine in *gallinaceum* malaria of chickens. Using a standardized quinine treatment, we found, as others have found, that there was less suppression of parasitemia and a greater mortality in splenectomized than in nonsplenectomized chickens. Our work indicates that during quinine treatment, three more or less independent antimalarial factors are active, viz innate immunity, acquired immunity, and the direct action of quinine on the parasite. Of the three factors, splenectomy only reduces the auxiliary action of acquired immunity.

All evidence at present points to the fact that relapse in malaria results from a lowering generally temporary, of immunity rather than a change in antigenicity of the parasites such as occurs in the trypanosomes. Thus, Coggeshall and Kumm (1938) found that the titer of the protective antibody drops just before a relapse in *knowlesi* infections in rhesus monkeys and rises markedly after recovery from relapse. Coggeshall (1943) also ascertained that the same blood can be used as the source of parasites and of serum in protection tests. This fact indicates that the parasite survives even with an unaltered antigenic structure. Although the belief has recently been strengthened that parasites survive during latency in some type of exoerythrocytic stage, it should always be kept in mind that no matter in what form the parasite persists, immunity against erythrocytic stages has to be reduced before erythrocytic stages can reaccumulate in the blood to produce a relapse.

Certain characteristics of malarial immunity such as antigenic potency amount of antigen absorbed, tendency to relapse and lability are closely related. Thus in relapsing infections such as *P. vivax*, *P. falciparum* and *P. brasilianum* the antigenic stimulation of the initial infection is minimal and is just sufficient to produce a comparatively mild and low grade immunity. Subsequent antigenic stimulation of relapses gradually reinforce this immunity until it is strong enough to eradicate the infection. At the other extreme in nonrelapsing infections, such as *P. catheherum* and *P. lophurae*, the antigenic stimulation even of mild infections seems to give rise to a high degree of immunity with few relapses. These interrelationships, already become apparent from the work of Lourie (1931), Simon

- Thomson, K J et al Amer Jour Trop Med 27 70, 1947  
Zuckerman, A Jour Infect Dis 77, 28, 1945

in understanding the epidemiology, pathogenesis, course, and treatment of the disease. It may also eventually yield helpful aids in diagnosis.

The study of malarial immunity has much wider implications than its possible immediate bearing on the human disease. The mechanisms are the same as those involved in immunity to other invading organisms. In fact, the malarial infections of man and, to a much greater extent, of animals, have yielded unique material for the study of certain aspects of immunity. Thus, the comparatively large size, synchronism of asexual reproduction and localization to the blood stream of some plasmodia have permitted the differentiation of parasitocidal and reproduction inhibiting effects of immunity by L. G. Taliaferro (1925) G. H. Boyd (1939), and the speaker (see W. H. and L. G. Taliaferro 1944 and 1947). The same attributes, together with the presence of malarial pigment which serves as a marker for a considerable time after the parasite has been digested, have facilitated the study of the cellular basis of immunity in a comparatively mild infection localized to the blood by Cannon (Hulligan, Bloom and the speaker (references previously cited)). Finally, the existence of sporozoites and exoerythrocytic and erythrocytic stages have lent themselves to an exceptional analysis of the action of immune factors against different stages of the same parasite by Huff and Coulston (1946). These and other less spectacular methods of attack have contributed to the general rationale of immune functions and mechanisms.

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ematic review containing a more extensive list of references will be published (1948a)

and in one of these cases parasites disappeared without any specific treatment (Raper, Wilson, and Wilson 1945).

The above findings refer mainly to the fighting tribes, recruited largely in highly malarious country. As the East African forces expanded, increasing numbers of the less warlike tribes, coming mainly from the high country, were recruited for the technical services. These services also absorbed numbers of more educated craftsmen and technical workers. In this group of African soldiers the reaction to malaria was quite different from that in the group already described, with the result that malaria rates were higher and individual attacks either troublesome or severe. A differential sickness rate for these men is not in general available, but in Ceylon the Medical Corps, Engineers, and Gunners had a malaria rate of 7 percent, three times that of the infantry. The average duration of fever under treatment was over 2 days in this group and some cases proved very refractory, possibly as a result of the tendency to take minimal amounts of treatment (when they could get it) for any slight attack of fever. Without specific treatment, fever continued for an average of 9.3 days, but the severity of symptoms sometimes compelled earlier specific treatment, even in this experimentally observed untreated group.

TABLE 1—*Malaria indices in the dry season*

| Area                           | Parasite rate |       |         | Spleen rate |       |         | Number examined |
|--------------------------------|---------------|-------|---------|-------------|-------|---------|-----------------|
|                                | 1-10          | 11-20 | Over 20 | 1-10        | 11-20 | Over 20 |                 |
| Wet lowlands                   |               |       |         |             |       |         |                 |
| Digo (Tanganyika)              | 91            | 58    | 45      | 85          | 63    | 39      | 3 000           |
| Sora (Uganda)                  | 69            | 66    | 42      | 75          | 41    | 16      | 86              |
| Mixed (Madagascar)             | 67            | 73    | 40      | 82          | 76    | 47      | 180             |
| Uplands                        |               |       |         |             |       |         |                 |
| Kiga (Uganda)                  | 63            | 39    | 28      | 72          | 70    | 78      | 150             |
| Nyiramba (Tanganyika)          | 70            | 26    | 16      | 87          | 72    | 80      | 237             |
| Mixed (Ethiopia) mainly Amhara | 19            | 31    |         | 72          | 78    |         | 190             |
| Dry lowlands                   |               |       |         |             |       |         |                 |
| Masaï (Tanganyika)             | 32            | 34    | 24      | 26          | 44    | 41      | 300             |
| Mixed (Somalia)                | 28            | 17    | 11      | 65          | 67    | 63      | 200             |

The gametocyte output of the two classes of soldiers was also very different, and in a mixed unit studied while on duty (Wilson and Wilson 1945), the gametocyte rate was only 1 percent in the immune group but 7 percent in the remainder. Similarly after treatment with short courses, the gametocyte rates were 7 to 10 percent in the immune and 25 to 32 percent in the susceptible group.

#### VARIATIONS IN TRIBAL ENDEMICITY

When surveys are made in the tribal areas of these various peoples,

homelands have resulted in substantially differing degrees of tribal

# SUSCEPTIBILITY TO MALARIA IN EAST AFRICANS

D BAGSTER WILSON, *Malariologist, Tanganyika Territory, and*  
MARGARET E WILSON, *from Muheza, Tanga, Tanganyika*

malaria is of greatest importance

## SUSCEPTIBILITY IN AFRICAN SOLDIERS

absence of attacks in Senegalese soldiers in Algeria, while the local population were suffering from repeated attacks. In our own experience, the malaria rate in West African troops who came to East Afr

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tribes stationed in Ceylon, the malaria rate was only 2.6 percent over a 9 month period, as compared with 25.6 percent in Europeans. Raper, Ogborn, and Wilson (1944) in a series of therapeutic trials, found that in their immune group of patients recovery had occurred almost as

specific antimalarial treatment. Several cases were observed in which dividing forms of *P. falciparum* were found without serious illness,



anopheline control being carried out in urban areas and around labour camps, and the total number of Africans protected by such measures is an appreciable fraction of the population. The immune status of the people concerned may be changed by this protection over a period of years, and children never acquire an immunity, as has been found, for example, on the copper belt in northern Rhodesia. This effect is enhanced by the increasing amount of antimalarial treatment that such people, and many others such as schoolboys and the better paid crafts men, receive. Whether or not this is a desirable development may be arguable, but it is an inevitable one, and must be accepted as one of the factors in the production of a mounting clinical malaria rate. Although in civil practice it is possible to obtain only an impression of the truth of this conclusion, military experience, because of its greater precision in recording disease incidence, leaves no doubt about it.

### EPIDEMIC AREAS

There is another far more important cause of a general increase in clinical malaria in East Africa, namely the invasion of areas hitherto malaria free or the general dissemination of malaria over districts previously only slightly affected by it. Such extension is almost wholly from lower to higher altitudes, and Garnham (1945) has already described epidemics occurring up to 8,500 feet (2,600 metres).

while in others it occurs for a short season during which a more or less severe epidemic is experienced, with its resultant effects of severe illness.

Elsewhere more fulminant epidemics occur. In the Kikuyu country, north of Nairobi, some hundreds of deaths resulted from such an

while no more than a few cases of local infection were recognized annually. Yet at the time of the epidemic severe illness was widely spread over most of the high uplands of which this district consists. The origin of the epidemic is somewhat obscure, but, as in Kigezi, there has been an increasing cultivation of valleys that had hitherto been sedge covered swamps, and now *Anopheles gambiae* is to be found nearly everywhere up to 6,000 feet, although usually in small

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been possible to observe only the dry season endemic indices. These

resistance to malaria, as shown in table 1. The aggressiveness of the tribe and the extent to which it was either pastoral or agricultural have been the controlling factors in determining the malariousness of its environment. Thus the Hamitic tribes which are generally immune to malaria are so because of their pastoral habit and preference for the open grasslands in the drier or upland country. At the less aggressive of the Bantu tribes were pushed up into the mountain forests where malaria has until recent years been absent, in contrast with the other agricultural tribes who took up the fertile well watered and most malarious lowland country. Whether these tribal differences in resistance to malaria are wholly due to their ways of life, or whether their ways of life were to some extent determined by a racial or tribal susceptibility to malaria, can not yet be decided. Some of the Nilotic tribes who live in very malarious country seem to be more susceptible to presumably alien strains of parasite when they go to other parts of the country.

TABLE 2.—Seasonal variations in parasite counts

| Place tribe             | Altitude | Months malaria season | Counts per 100 mosquitoes |                |            |                |
|-------------------------|----------|-----------------------|---------------------------|----------------|------------|----------------|
|                         |          |                       | Children                  |                | Adults     |                |
|                         |          |                       | Off season                | Malaria season | Off season | Malaria season |
| Dira (Tanzania) (Ka)    | 300      | 8-10                  | 200                       | 190            | 74         | 15             |
| Dura (T. ganda)         | 2,000    | 8-10                  | 170                       | 140            | 23         | 41             |
| Dura Suani (Madagascar) | 100      | 6-8                   | 150                       | 190            | 12         | 85             |
| Nyirmita (T. aganyika)  | 4,800    | 6-8                   | 120                       | 300            | 30         | 20             |
| Rika (T. ganda)         | 6,000    | 4-6                   | 84                        | 73             | 26         | 150            |
| Rikwa tribes (Somalia)  | 300      | 2-4                   | 35                        | 100            | 35         | 150            |
| Romali (Somalia)        | 1,000    | 2-3                   | 30                        | 410            | 10         | 420            |

There is, whatever the circumstances of its origin, a great range of difference between different areas, as is shown in table 1. This difference is more clearly evidenced by the seasonal variations found in the parasite counts shown in table 2, which also gives the approximate annual periods of malaria transmission. The frequency of infection seems in general to depend mainly on the duration of the malaria season, although of course anopheline numbers and infectivity are factors that may vary independently and become, more rarely, the determining influences. In general, therefore, the shorter the transmission season the greater the difference between parasite infestation in the malaria and off seasons, in conformity with the greater susceptibility to malaria.

#### EFFECTS OF ANOPHELINE CONTROL AND TREATMENT

There is an increasingly large proportion of East Africa in which frequency of infection is being modified by the more or less effective

But the vast majority of the Somali only encounter malaria from time to time, and spleen rates range between 2 and 30 percent, except during an epidemic when they rise abruptly to 50 percent or more. The parasite rate usually found under such circumstances is of a much lower order, 18 to 20 percent in the example shown in table 4, are suffering

1 malaria ex  
hibits a degree of virulence to Africans that contrasts greatly with the more commonly found premunition in most of tropical Africa. It is probably true, however, that some 3 to 4 million people in East Africa alone may be subject to this type of risk, and so it provides a problem of some magnitude and one which has an increasing importance.

### MEASUREMENT OF MALARIA

Although we have repeatedly referred in the past to the characteristic variations in the endemic indices that emerge under varying conditions of malaria transmission, we make no apology for reverting to this question, since the paper go some way towards may be encountered. The suitable measurement of the results of frequent or infrequent infection, and this holds at least over a very wide area of the African continent.

The first and obvious requirement is that measurement of malaria in man must be related to season. Evident as this may be, it is a stipulation that is too often ignored. In fully hyperendemic areas there is evidently little difference between one season and another, but in the presence of other grades of endemicity, wide and informative increases may be found in both spleen and parasite rates during the malaria season. If possible, therefore, measurements should be made both in the dry season and at the height of the transmission season.

The restriction of observations to children, as is commonly done, and which may of course be inevitable in some places, leaves out of account much information that may be of great, or even critical, importance in the initial assessment of endemicity. A high degree of susceptibility in adults is shown more particularly during the transmission season, not only by the attack rate but also by a spleen rate that approximates that of children. The spleen rate in adults will nearly always be somewhat lower, owing to the greater difficulty experienced by the examiner in palpating the adult abdomen, particularly that of a muscular male. However, this difficulty does not complementary  
ria, we have made a practice of  
s, and although this is a crude

# V MALARIA

varied widely, as shown in table 3, and in some places there is an apparent residuum from the epidemic, which was most severe at levels over 5,000 feet. The inhabitants of Mbulu, of Hamiti, are now also agriculturalists, although their cattle are still of interest to them. To such people malaria is a serious, and in critical, handicap. This epidemic did, in fact, interfere with cotton and other communal activities.

TABLE 3—Malaria indices in Mbulu

| Place     | Altitude (feet) | Spleen rate |         | Parasite rate |         |
|-----------|-----------------|-------------|---------|---------------|---------|
|           |                 | 1 to 20     | Over 20 | 1 to 20       | Over 20 |
| Tlawi     | 6,800           | 2           | 1       | 4             | 28      |
| Dongobesh | 7,000           | 5           | 10      | 28            | 15      |
| Fodasak   | 8,300           | 16          | 18      |               |         |
| Habati    | 4,800           | 21          | 23      |               |         |

The almost wholly pastoral Somali Hamites, living in the arid lands around the Horn of Africa, have been subject to malaria epidemics for many years. Here malaria is almost wholly seasonal owing to the small amount and brief duration of rainfall. But when rain does fall in adequate quantities, *A. gambiae* breeds in vast numbers, and adults may average 100 or more per small hut. The result is to incapacitate a large proportion of the population and sometimes to kill many of them, since they will not, for their own sake, abandon the best grazing for their herds in the immediate vicinity of the dangerous rainpools. There is a very circumscribed, moderately endemic area in which spleen rates ranging between 50 and 75 percent are found, and a moderate degree of resistance to malaria is developed.

TABLE 4—Malaria indices in British Somaliland

| Malaria Indices in British Somaliland |           |             |               |               |               |  |  |
|---------------------------------------|-----------|-------------|---------------|---------------|---------------|--|--|
| Area                                  | Age group | Laisi       |               | Abel Qadr     |               |  |  |
|                                       |           | Spleen rate | Parasite rate | Spleen rate   | Parasite rate |  |  |
| Epidemic                              | 1-10      | 75          | 20            | 35            | 16            |  |  |
|                                       | 11-20     | 30          | 20            | 34            | 32            |  |  |
|                                       | Over 20   | 42          | 7             | 28            | 11            |  |  |
| Examined                              |           | 100         |               | 94            |               |  |  |
|                                       |           | No epidemic |               | Epidemic      |               |  |  |
|                                       |           | Spleen rate | Parasite rate | Spleen rate   | Parasite rate |  |  |
| Non-epidemic                          | 1-10      | 81          | 4             | 50            | 30            |  |  |
|                                       | 11-20     | 72          | 7             | 45            | 14            |  |  |
|                                       | Over 20   | 17          | 1             | 30            | 20            |  |  |
| Examined                              |           | 20          |               | 100           |               |  |  |
|                                       |           |             |               | Post epidemic |               |  |  |
|                                       |           | Spleen rate | Parasite rate | Spleen rate   | Parasite rate |  |  |
|                                       |           |             |               | 38            | 8             |  |  |
|                                       |           |             |               | 34            | 5             |  |  |
|                                       |           |             |               | 30            | 6             |  |  |
|                                       |           |             |               | 10            |               |  |  |

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It referred to this problem. The malaria incidence in hyperendemic regions, as measured by spleen rate and parasite rate, is different in

In Indonesia the spleen rate runs up to about 60 percent in adults. In

Negro children the spleen rate is as high as in Indonesians, but in adults it is much lower, the parasite rate in toddlers comes near to 100 percent and in adults it never gets under 20 percent. Schuffner and myself thought that this difference was due to a racial difference between Malays and Negroes. But Bagster Wilson said it was due to the more intense infection to which the Negroes were exposed. This engenders a more complete immunity which, in its turn, causes the spleen rate in adults to subside.

In New Guinea the numbers of the *punctulatus* group cause a heavy malaria infection. Nevertheless the spleen rate among the Papuan adults is supposed to be as high as among the Papuan children. So I hoped to be able to prove Dr. Wilson in the wrong by showing the

high, as high as the Negroes', and far above what we are wont to see in Indonesia, although the incidence of heavy infections is less than in Negroes and more like that in Indonesians. But their adult spleen rate is distinctly less than in their children, although the difference is less than in Negroes.

On the whole, the Papuans stand in between Indonesians and Negroes. That is just what Dr. Wilson's theory would require. And so I am inclined to believe that he was right and we were wrong—although I concede that these data do not offer a completely satisfactory proof that his view is right.

Dr. L. J. CHWATT (Nigeria). The series of recent, still unpublished data collected in a hyperendemic area in West Africa may serve as a circumstantial though strong evidence of the existence of an inherited, passive, immunity to malaria and may emphasize its importance in modifying the course of an initial, untreated infection of African babies with *P. falciparum*.

These data are being still collected from 460 African infants investigated not by random sampling but by a clinical and haematological examination repeated every 2 to 4 weeks. Detailed and tabulated data are now available for 91 babies, seen regularly from 1 to 2 weeks of life to 12 to 14 months.

The average anopheles infective density of the area is such that

## V MALARIA

method that should not be regarded as a precise measurement, seemed to us to reveal qualitative differences that could not be attained in any other way with an approaching degree of certainty. In general, progress in the study of the epidemiology of malaria requires that there should be a more general acceptance of the different methods of malaria measurement than that of practical and useful method of spleen measurement than that of Hackett (1944), and its wide adoption as a routine would provide comparable data. But this is only one of the indices used, and an appeal is made for some formula to be agreed for all measurement of vectors as well as of human malaria.

At the present time it is rarely possible to make adequate comparisons between the data observed by different workers (quite apart from human variations in the observer), but it might well be that for example parallel records of the reaction of the Negro to infection in the American Continent and his reactions in his homeland would be of both academic and practical value. To summarize, our observations indicate the following criteria of the different levels of malarial endemicity in East Africa.

**Hyperendemic**—Spleen and parasite rates falling with age but constant from season to season except in babies, little change in the parasite infestation or count.

**Endemic**—Parasite rates lower than in the former group but still falling with age, spleen rates as high as in the hyperendemic group and not falling greatly with age. All rates rise to a varying extent during the malaria season but the parasite infestation rises most markedly.

**Epidemic**—Parasite and spleen rates may even rise with age the latter being always higher than the parasite rate, although they both show a big seasonal variation, and parasite infestation is many times greater in the malaria season.

Average spleen size generally follows the spleen rate except in the epidemic areas, where it tends to increase with age.

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## ABSTRACT OF DISCUSSION

H. SWELLENGREBEL (Netherlands) I want to say a few words  
Wilson's paper. Not on the one he read just now, but 10 years  
the Amsterdam Congress of 1938.

Whether this humoral passive immunity is transplacental or provided with the mother's milk remains to be shown. It may be of some interest to remember here Culbertson's experiments with mice inoculated with *T. duttoni* and rats infected with *T. lewisi*. The litter from

I should like to add that in 1945 Garnham completed an infant survey in the Kavirondo around Kisumu in West Africa. The results of his work, which is earlier than mine and will shortly be published, are extremely similar to those quoted above.

One final word—the fact that a newborn African baby exhibits a good deal of passive, inherited immunity to the malaria parasite decreases obviously the value of the newborn first infection index, used as a yardstick of the amount of transmission in hyperendemic areas in Africa.

The frequency distribution curve of parasite densities calculated as a geometric mean for each group has revealed that in three fourths of all cases the density of the first infection is not high and varies between 100 and 1,000 parasites per cubic millimeter. Only in 4.5 percent of cases the initial density was 10,000 per cubic millimeter or higher.

This initial parasitaemia is invariably high when first seen in the fourth quarter of the first year of life.

It was possible to distinguish four different trends in the course of the initial untreated infection in African infants. In about 40 percent of our infants the low initial parasite density lasts for 2 to 4 months with variations and then decreases considerably or even becomes negative. In 20 percent of our infants this low initial parasite density rises steadily or abruptly to 10,000 or over and maintains its high values. Clinically there seems to be little evidence of signs of overt malaria in those infants that exhibit low or moderate parasitaemia. In cases with parasitaemia of over 10,000, 85 percent of infants exhibit most of the signs of overt malaria.

Two groups were compared: babies that were permanently parasite negative during the first 9 months of life and babies that had parasites in their blood at any time during the same period.

It was found that fever above 100° F, convulsions, minor ailments

However, symptoms of severe overt malaria were surprisingly infrequent and the comparative mortality of both groups was only slightly higher than expected.

infancy

This investigation is being pursued. Its temporary results confirm what was said with regard to human malaria about 10 years ago by Schulling, by Barber and Rice, by Hackett, and by Clark. It seems that infants born from highly immune African mothers inherit a good deal of passive, human immunity which shows itself

than the infancy which from the malaria point of view is the crucial phase.



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### PIGMENTS

The salient characteristic of all the conditions is the presence of haemoglobin and/or its derivatives in the plasma. This is due to the liberation of haemoglobin from the red cells by the action of various factors. The haemoglobin molecule is a complex of four polypeptide chains (two  $\alpha$  and two  $\beta$ ) and a central heme group. The heme group consists of a central iron atom coordinated to four nitrogen atoms in a porphyrin-like ring. The iron atom is also coordinated to a proximal histidine residue and a distal water molecule. The heme group is responsible for the oxygen-carrying capacity of haemoglobin. In the conditions mentioned above, the haemoglobin molecule is released from the red cells and enters the plasma. This is known as haemolysis. The released haemoglobin can be oxidized to methaemoglobin, where the iron atom is in the ferric state (Fe<sup>3+</sup>) instead of the ferrous state (Fe<sup>2+</sup>). Methaemoglobin is unable to carry oxygen. The presence of methaemoglobin in the plasma is a sign of severe haemolysis. It is also found in the urine. The weight and diameter of the haemoglobin molecule are such that it is not filtered by the glomerulus. However, in severe cases, it can be filtered and appear in the urine. The injection of haematin into man, monkeys, and rabbits results in an increased excretion of faecal porphyrin and suggests that methaemoglobin is removed from the circulation by the liver and there converted into porphyrin.

Although methaemoglobin is never found in the plasma of black-water fever, it is frequently present in the urine, and its appearance in the urine bears no relation to methaemalbumin in the plasma or to the pH of the urine (Foy and Kondi 1938). Methaemoglobin does, however, occur in the blood of some of the other haemolytic conditions such as that which may follow malaria, sickle-cell anaemia, or thalassaemia.

The presence of methaemoglobin in the blood is one of extreme importance as it may have a fundamental bearing on the metabolism of the red cell. It has been shown by a number of workers that methaemoglobin is present in small amounts in normal blood, and the work of preventing too great an oxidation of oxyhaemoglobin to methaemoglobin is of great importance.

# BLACKWATER FEVER AND THE INTRAVASCULAR HEMOLYSES

HENRY FOX, *Wellcome Trust Research Laboratories, Salomon*

## INTRODUCTION

The central problem of blackwater fever -

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an understanding of the phenomenon must include a study of other haemolytic conditions such as paroxysmal nocturnal haemoglobinuria, bean haemoglobinuria (favism), familial and acquired haemolytic jaundice, march haemoglobinuria, the haemolyses that sometimes follow incompatible transfusions, and the administration of sulphonamides and other drugs.

In all these haemolytic diseases, it will be found that they have certain fundamental resemblances and differences depending on the type, degree, and rapidity of the haemolytic processes. The indirect van den Berg will generally be raised, and this will be an index not only of the degree of blood destruction but also of any unpaired ability to remove the products of haemolysis, perhaps connected with the R E S in general and the liver and spleen in particular. As the haemolysis proceeds, Schum's test will become positive, and methaemalbumen will appear in the plasma spectroscopically. With increasing red-cell destruction these will be accompanied by haemoglobinaemia, and when the kidney threshold for haemoglobin has been reached, haemoglobin will appear in the urine. In some of these conditions (blackwater fever, favism, drug haemolyses, and incompatible transfusion) greater or less degrees of renal impairment may develop and lead to nitrogen retention, oliguria, and ultimately, in some cases to anuria.

The common denominator in all these conditions is the passage of haemoglobin from the red cells into the plasma. Whether the red cells lose all their contents of haemoglobin by a process of physical destruction of the cell membrane or merely lose part of it as a result of changes in the permeability of the cell wall is a question that has so far not been fully answered, it appears to vary in the different conditions.

The differences between the various haemolytic conditions are just as striking as their resemblances. For example, the pronounced spherocytosis in familial haemolytic icterus and abnormal osmotic fragility is in sharp contrast to blackwater fever where changes in the surface and volume measurements of the red cell are minimal and osmotic fragility not altered, the strange phenomenon of splenectomy in haemolytic jaundice which produces cessation of the periodic haemolyses but leaves the spherocytosis and abnormal osmotic fragility un-

is due to physical disintegration of the cell, or to changes in the permeability of its wall that permit the whole or part of the haemoglobin to escape, is at present not known for all the various types of intravascular haemolyses. There can be little question that in black water fever red cells in various stages of disintegration and phagocytosis can be seen, but whether they have lost all or part of their haemoglobin before breaking up is not easy to determine. In any haemolytic process there may be a certain proportion of cells that lose no haemoglobin, another portion which may lose only a small percentage, and still another portion that lose greater amounts. It is then unsatisfactory to postulate that a certain number of cells lose all their haemoglobin, or that all the cells lose only part of their haemoglobin content, conditions may vary in the different diseases, and perhaps in the same disease at different times. It is, for example, well known that red cells can lose the major portion if not all their haemoglobin and still remain intact for some time. In such cases there is evidence that the haemoglobin leaves the cell first, and later the cell disintegrates and is phagocytosed.

The problem of the passage of the large molecule of haemoglobin through the cell wall is one of fundamental importance in all haemolytic conditions, and we have recently been investigating it from the point of view of cation permeability using the radioactive isotopes of sodium ( $\text{Na } 24$ ) and potassium ( $\text{K } 42$ ) in various haemolytic conditions of man and animals<sup>1</sup>. Svedberg (1930) has postulated that the

of 68,000 may pass  
into 17,000 units and

Thus, however, has been criticized by Schmidt (1932). It appears that the red cell is somewhat intolerant of exchanging cations to any great extent, nor is this selectivity greatly changed in the haemolytic conditions that we

sium is the dominant cation in  
dist sodium is the cation of the

This raises the interesting problem of changes in cell permeability to cations at different stages of the life cycle of the cell. The red cell's high potassium content must have been acquired at some time during its development, the cell later appears to lose this ability to exchange potassium to any important degree, but having once acquired it, it may maintain its equilibrium by means of minimal exchanges. Davson and Danielli (1938) showed that there is a prehaemolytic escape of potassium from the cell in vitro in the presence of various haemolytic agents, and Ponder (1947) has considerably extended this work using flame photometry.

Evidently there is, at least in vitro, a prolytic escape of potassium from the red cell when in hypolytic suspensions of various haemolytic

<sup>1</sup> In press

globin is accomplished by some enzyme system related to glutathione (Morrison and Williams 1938, Foy and Kondi 1944), and drugs as plasmochine, nitrites, aniline, acetanilide sulphonamides etc., upset this enzyme system and allow the formation of intracorporeal methaemoglobin. The well known action of such reversible oxidizing agents as methylene blue and ascorbic acid is of great interest in this connection.

### KIDNEY FUNCTION

During the past few years a great deal of work has been carried out on the question of the causes of renal failure in blackwater fever and the other intravascular haemolyses. The consensus of opinion seems to favor the view that the oliguria and anuria that sometimes develop in conditions of intravascular haemolysis cannot be explained by the simple process of blockage of the renal tubules with precipitated products of haemoglobin in an acid filtrate (Foy and Kondi 1943, Macgrath and Findlay 1944, Peters 1945, etc.). It appears that the factors of greatest importance are changes in glomerular filtration and tubular reabsorption, incident on redistribution of the blood supply through the kidney (Macgrath 1944, Trueta 1946, Tomb 1942, Flink 1947). Anoxia and ischemia of the organ are, no doubt, of great importance in bringing about changes in function that will lead to upsets in filtration and reabsorption. It has been shown that dehydration accompanied by haemoglobinemia is also an important entity in renal failure (Lahliou 1948). The glomerular filtrate has been shown by rather (1948) and Yuile (1941), not to be protein free in normal states, some or all of this protein in the filtrate is taken up by a process of athrocytosis in the tubules, and if abnormal amounts of protein are present, interference of the flow of filtrate through the nephron may result and upset glomerular filtration. Clearances of inulin and diodrast have been found to be changed in haemoglobinuric states, again indicating filtration and reabsorption changes. There seems to be agreement that if dehydration precedes accompaniment, or follows haemoglobinemia renal damage and failure are more likely since the kidney works with a reserve of some 60 to 80 percent, blockage would have to be very extensive before it could produce anuria (De Navasquez 1941), and there is little evidence that such extensive blockage occurs in anuric cases that have come to post mortem (Foy 1943). What factors are responsible for the renal blood redistribution in these haemolytic conditions and the physiological disturbances that follow is at present a matter for speculation, Hesse and co (1933) have suggested as the primary cause renal spasm, a rise to cortical ischemia, blood redistribution, and differential anemia.

### CAUSES OF HAEMOLYSIS

In its broadest aspect, haemolysis may be defined as passage of haemoglobin out of the red cell. Whether this escape of haemoglobin

osmotic fragility and minimal spherocytosis. Transfusion of red cells from cases of blackwater fever taken during the height of the haemolytic crisis have a very much reduced survival time in normal individuals, but the life span increases as the length of time from the haemolytic crisis increases. Red cells from normal individuals transfused into acute cases of blackwater fever are lysed just as readily as are the patient's own cells (Foy, Kondi, Rebelo, and Soeiro 1945). Further large amounts of plasma transfused from fulminating cases of blackwater into normal recipients produce no untoward effects. These facts . . . blackwater fever there is not only . . . destroys all cells that come into c . . . y be some red cell abnormality in addition, as shown by reduced survival of patients' red cells in normal individuals. It is, however, somewhat startling that plasma taken during the height of a haemolytic crisis produces no effect in the recipient. Findlay and Markson (1947) have described the results of injecting malaria blood into recovered cases of blackwater fever with the production of haemolysis in three out of six cases. Injection of normal blood produced no effects, nor did injection of the same malaria blood into normal individuals.

Although blackwater fever . . . normal osmotic fragility to saline, their fragility to cells from cases of familial . . . 1943). Bergenhem (1936) and Fairbairns (1939) suggested that . . . molytic conditions . . . nating blood in an . . . cells allowing lysis . . . ork (1936) on the

anatomy of the spleen tended to add support to this view, but the more recent work of Mackenzie et al (1940) and Whipple (1941) on

Ponder  
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a straightforward one is shown by the fact that intravenous injection of large quantities of lysolecithin in baboons fails to produce any sign of haemolysis or even spherocytosis (Foy and Kondi 1943). This may be due to inhibition by plasma proteins or to other unknown factors. Kellaway (1933) and Holden (1935) have shown that in snake venom haemolysis lecithinase is the important factor. It is possible that lecithinase, acting on a substrate of lecithin in plasma or cells, splits the lecithin into an unsaturated fatty acid and lysolecithin and may . . . of import  
membran

agents The former view that the red cell wall is impermeable can no longer be substantiated so far as in vitro work concerned and there is some possibility that minimal exchanges may place in vivo Loss of potassium from the cell can be and is replaced by sodium with minimum swelling of the cell The sodium taken generally greater than the potassium lost There appears to be a relation between sphering and potassium loss from the erythrocyte the loss of haemoglobin from the cell is always less than the loss of potassium, and in completely haemolysed systems there is always a haemoglobin remaining in the red cell ghost than in the surrounding medium

A controlling influence in the loss of potassium from the cell is the presence of plasma of which the albumen fraction is the important entity, and no doubt this will be a factor in in vivo states. In vitro systems the loss of haemoglobin is an all or none phenomenon but this is certainly not the case with potassium There is still some doubt as to what proportion of the potassium is lost from the cell. It appears that in many cases the potassium exchange may be of a low order, although there is evidence that in muscle cells in a potassium free perfusate all the potassium leaves and can be replaced by sodium

In the haemolytic conditions that we have so far investigated by means of radioactive isotopes it seems that there is very little if any change in cation permeability when compared with normal states. This work is being continued and will be reported later in detail. Substances like CN, CO, or methane which inhibit metabolic processes do not affect the loss of potassium from the cell, nor is loss affected by methylene blue. It seems then that the retention of such large amounts of potassium is not controlled by usual metabolic processes

The problem of what causes the sudden destruction of the red cells in blackwater fever still remains. In familial haemolytic icterus it has been shown that red cells from the patient transfused into normal individuals have a reduced life span (Dicke and Mollison 1943), whilst normal cells transfused into cases of familial jaundice have a normal life span. Removal of the spleen in such patients stops the periodic haemolytic crises but leaves the abnormal osmotic fragility of saline and spherocytosis unaltered. This seems to indicate that in his disease there are two factors at work an abnormality of the red cells, as well as some factor that is dependent on the presence of the spleen. In acquired haemolytic jaundice, on the other hand, the cells transfused into normal recipients had a normal survival time. In the patient transfused into normal individuals transfused into patients with acquired haemolytic jaundice have a shortened survival time indicating that in acquired haemolytic jaundice there is probably some inhibiting haemolysin (Loutit 1946)

The situation in blackwater fever is different there is no changed

the possibility should not be completely ruled out. Recently Jacobs (1947) reviewed the evidence in connection with antierythrocyte activity and reports a number of his own experiments, and concludes that so far as his work is concerned there is little to support this hypothesis, although he admits that there was no evidence that the material that he employed provoked any sort of antierythrocyte response.

### PARASITES

There is no evidence that specific parasites, other than malaria, can be regarded as important in the genesis of blackwater fever. Nor have haemolytic strains of malaria been shown to exist in the sense that they can produce a sudden and profound haemolysis such as is characteristic of blackwater (Toy and Kondi 1936, 1941). Strains of *P. falciparum* that are highly drug resistant cannot be excluded as a factor, but the precise niche that this parasite occupies in the blackwater fever picture is by no means clear. Cases of blackwater have been reported in infections where *P. vivax* was said to have been the only parasite, but many of these reports are not sufficiently discriminating to be watertight evidence. The works of Fairley (1945) in the Pacific showed that blackwater fever was rare after the introduction of atabrin prophylaxis, and he suggested that this may be due to the wiping out of the falciparum infections.

### DRUGS

Drugs occupy a controversial position in the genesis of blackwater fever. The correlation coefficient between the last dose of quinine

to be of value in the treatment of malaria may be seen in a blackwater fever. Many cases have been reported after atabrin. (Toy and Kondi 1937, Manson Bahr, Abbott 1946, etc.)

taken in very large amounts in Greece. During the war it was not available, and atabrin was substituted. How far the fall in both

It is interesting  
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undoubted cases of blackwater fever, but although there is no evidence that he had quinine, he may have had some other antimalarial

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metabolism, they do not think that circulating haemolysins are responsible, nor do they consider that the changes in osmotic fragility conditions, spherocytosis is accompanied by the cells as well as by changes in their areas and volumes, but these changes are not invariably linked to variations in osmotic fragility to saline.

In blackwater fever it has been shown that the

affects the distribution of haemoglobin in the blood, so that in paroxysmal nocturnal haemoglobinuria and possibly also in blackwater fever there is a certain percentage only of red cells that are abnormal, and that therefore when the blood count is high the evidence of haemolysis is more pronounced than when it is low, evidenced by the fact that haemoglobinuria starts only when the red cell count has reached the higher levels, at low blood counts the

in subjects infected with *P. knowlesi* infections, and that the activity of this substance is greatly enhanced by haemolysis.

It is suggested that the antigen as a result of malaria infection, which in the presence of complement

in cases of blackwater fever, as also in recovered cases, Coombs test is consistently negative as also it is in paroxysmal nocturnal haemoglobinuria.



# THE UNITED STATES PUBLIC HEALTH SERVICE MALARIA CONTROL PROGRAM IN THE PHILIPPINES

FRANCISCO J. DY, M. D., M. P. H. *Consultant in Malaria and Chief of the Malaria Control Division, U. S. Public Health Service in the Philippines*

The United States Public Health Service malaria control program in the Philippines was started in April 1946, as part of the program designed to assist the Philippine Commonwealth Government in the rehabilitation of its quarantine and public health services, through an appropriation made by the United States Congress in December 1945. This program of 1946 passed by provides for the

services and facilities throughout the Philippines up to June 30, 1950.

Surveys made immediately following the liberation of the Philippines from the Japanese revealed that malaria was one of the greatest public health problems of the country. Prior to World War II, it was estimated that there were about 2 million cases of malaria throughout the Philippines every year. It was conservatively estimated that the incidence of the disease had more than doubled due to lack of food and medicine, deterioration of public health service, displacement of population, and lack of adequate hospital facilities during the Japanese occupation. When industries producing lumber, sugar, copra, and other commodities resumed operation subsequent to the liberation of the country, 30 to 50 percent of the laborers in most malarious areas were usually absent every day, and fertile lands lay idle due to malaria. It was evident that control of the disease was urgently and vitally necessary for humanitarian reasons as well as for rehabilitation purposes. It was also evident that the Malaria Control Section of the Philippine Bureau of Health with only five malaria control units and a meager appropriation would be unable to cope with the tremendous public health problem that confronted the newly liberated country.

Based on these realistic considerations, the United States Public Health Service has worked out a malaria control program with the following objectives:

- 1 To effect a successful joint United States Philippine malaria prevention and control program.
- 2 To render immediate relief to malaria victims by setting up a free laboratory service, combined with home and dispensary treatment of cases.
- 3 To eradicate or control the malaria vector by the institution of temporary or permanent control measures.
- 4 To institute a malaria education campaign for the masses.

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assistant surgeon general in charge through the director of field operations. The consultant has a staff of field supervisors including two entomologists, a malarialogist, a parasitologist, and a malaria engineer. These supervisors make periodic inspections of the units and coordinate the work as a whole. Each malaria unit is composed of two medical officers (malarialogists), two parasitology technicians, two entomology technicians, and a malaria engineer who serves one or two other adjoining units which may be without an engineer. It has been the practice to reduce the number of malarialogists in a unit to one when the malaria situation in the area of responsibility of the unit has been brought under control. In addition to the normal complement of each unit, up to 30 laborers are hired locally on a daily basis to assist in the vector control projects. Each unit is provided with a  $\frac{1}{2}$  ton or  $\frac{3}{4}$  ton truck, microscopes, sprayers, antimalaria drugs, and other equipment and material.

The functions of the malaria units are briefly as follows

**A. Malaria survey**

- 1 Collection and evaluation of morbidity, parasite, inoculation, and spleen rates and indices as well as larva and adult densities of mosquito vector
- 2 Determination of the presence and incidence of malaria infection in communities
- 3 Study of the topography, meteorology, demography, and socio economic factors in malarious communities.

**B. Malaria relief**

- 1 Free treatment of malaria patients in dispensaries and in homes
- 2 Laboratory service for examination of blood smears
- 3 Advisory and consultative service to patients and physicians

**C. Malaria control**

- 1 Planning construction, repair, and maintenance of automatic siphons and dams with sluice gates
- 2 Larviciding by means of sprayers and DDT treated saw dust
- 3 Brushing and clearing of streams, channelling, ditching subsoil drainage, etc
- 4 Other vector control measures.

**D. Malaria educational campaign**

- 1 Lectures and conferences with medical societies, hospital personnel, and private medical practitioners
- 2 Lectures, demonstrations, and conferences in schools, public meetings, house to house visits, town fairs and carnivals etc
- 3 Exhibition of moving pictures on malaria prevention and control

5 To demonstrate to local landholders, corporations, and interested individuals, that malaria can and should be controlled as to convince them of the desirability of continuing the work of malaria control units in their farms and concessions, even if government assistance be discontinued

6 To carry out such research as may be indicated to improve current methods of treatment and control

One of the greatest problems encountered in initiating the program was the scarcity of adequately trained personnel. As the program aimed at improving and augmenting the over all malaria control facilities of the country, it was not deemed advisable to deplete the Philippine Bureau of Health of its malaria control personnel for the purpose of activating new United States Public Health Service units. For the same reason, it was not considered wise to hire foreign malaria control men, who would invariably leave the country after the program was ended. The personnel problem was solved by training Filipino workers, not only in the mechanics of malaria control and methods of treatment, but also in administration and public relations. During the past 2 years, over 200 such men have been trained and assigned as malariologists, entomologists, parasitologists, malaria engineers and technicians in the 20 malaria units and 4 entomological survey teams which are now operating in malarious provinces.

The 20 malaria units are now operating in the following provinces: Agusan, Bataan, Cagayan, Cotabato, Davao, Ilocos Sur, Laguna, Marinduque, Mindoro, Misamis Oriental, Negros Occidental, Negros Oriental, Nueva Vizcaya.

Since these 20 units cannot possibly cover all the malarious areas in the Philippines in the 4 years during which the program is supposed to operate, great care was exercised in selecting the areas where the units were assigned. In this selection, the following factors were taken into consideration:

- 1 *Population and development*—Incidence of malaria, density of population, and availability of communication facilities, roads, etc., in the area which will enable the units to render service to the greatest number of people in relation to the amount of money to be spent for treatment and other control measures.
- 2 *Socio economic status*—Relative importance of the area as a source of national revenue and its importance with reference to essential food production.
- 3 *Permanency of control measures*—Possibility of permanent control measures being installed and maintained by agricultural and industrial enterprises after practicability is established and demonstrated by the malaria units.
- 4 *Malaria Control Division of the United States Public Health Service*—Malaria Control Division of the United States Public Health Service which has been charged with the responsibility of carrying out the program is headed by a consultant who is responsible to the

The Malaria Control Division also directs and supervises the mosquito control work in and around the International Airport as an aid to the Philippine Bureau of Quarantine

TABLE 1—*The mean epidemic rate parasite and spleen indices in 80 malarious barrios in different parts of the Philippines before and after 10 to 14 months of quinacrine hydrochloride treatment<sup>1</sup> of cases and vector control work in 1946-47 Total population 32 631*

| Year surveyed                                       | Epidemic rate | Parasite index | Spleen index |
|---|---------------|----------------|--------------|
|   | Percent       | Percent        | Percent      |
| 1946—Before control                                 | 4.03±0.33     | 29.61±1.48     | 40.58±1.89   |
| 1947—After control                                  | 1.78±.36      | 17.80±.25      | 18.81±.14    |
| Difference of mean and standard error of difference | 2.25±.074     | 10.81±2.23     | 23.77±2.83   |

<sup>1</sup> Standard quinacrine hydrochloride treatment 4 tablets (0.10 gram per tablet) t. i. d. on day 1 and 1 tablet t. i. d. on days 2 through 7

Table 1 shows the mean epidemic rates and parasite and spleen indices in 80 malarious barrios (subdivisions of municipalities) in different parts of the Philippines before and 10 to 14 months after the institution of control measures. It will be noted that the reduction in the mean rate and indices is statistically significant.

| Year surveyed                                       | Epidemic rate | Parasite index | Spleen index |
|---|---------------|----------------|--------------|
|   | Percent       | Percent        | Percent      |
| 1947—Before control                                 | 9.81±1.29     | 33.37±1.38     | 30.6±1.58    |
| 1949—After control                                  | 2.44±.29      | 9.17±.79       | 8.1±1.11     |
| Difference of mean and standard error of difference | 7.07±.5.99    | 24.20±2.34     | 21.91±2.60   |

<sup>1</sup> Standard quinacrine hydrochloride treatment 4 tablets (0.10 gram per tablet) t. i. d. on day 1 and 1 tablet t. i. d. on days 2 through 7

Table 2, which represents an analysis of the rates and indices in 59 other malarious barrios before and 10 to 14 months after the institution of control measures, shows a significant reduction in the mean parasite and spleen indices. While the reduction in the mean epidemic rate in this group is not statistically significant, the fact should be considered that this rate is easily influenced by the concomitant presence in a malarious locality of other febrile diseases, such as upper respiratory infections, which may be included in the census as malaria. In the course of malaria surveys in endemic areas, obscure febrile disorders are generally considered as malaria unless proven to be another disease.

It is pertinent to state that the 139 barrios studied in tables 1 and 2, with a total population of 85,152, are but a small representative sample of the hundreds of other barrios where malaria units have been operating. It is unfortunate that lack of sufficient data con-

In addition to the 20 malaria units 4 entomological survey teams have recently been activated. Each of these teams is headed by an entomologist with two entomology technicians. The teams are provided with a minimum amount of equipment and supplies to enable them to move fast and to permit the personnel to live and work in hilly or mountainous places where their services may be needed. The teams and malaria units work independently, but the respective heads coordinate their work if they are stationed in the same general vicinity. The teams were activated in order to carry out important entomological studies such as the determination of the presence of vectors other than *Anopheles minimus flavirostris* without slowing down the control work of the malaria units.

The following is a statistical resume of the salient activities of the units from April 1946 through March 31 1948.<sup>1</sup>

|    |  |           |
|----|--|-----------|
|    |  | 290 281   |
|    |  | 53 434    |
|    | Total attacks treated in homes                                   | 270 715   |
| 2  | At dispensaries  |           |
| a  | Number of new cases  | 107 280   |
| b  | Number of relapses or reinfections                               | 101 502   |
|    | Total attacks treated in dispensaries                            | 208 839   |
|    |  | 423 567   |
|    |  | 104 086   |
|    |  | 578 503   |
| c  | Average number of dispensaries operated per month                | 186       |
| D  | Number of persons whose blood smears were examined for malaria   | 200 24    |
|    |  | 101 770   |
|    |  | 29        |
|    |  | 684 884   |
|    |  | 141 193   |
|    |  | 710 687   |
|    |  | 107 089   |
|    |  | 09 080    |
|    | tions  | 561 607   |
| II | Total number of persons  | 1 700 157 |
|    |  | 24        |
|    | repaired   | 38        |
| 3  | Total number of automatic sphygmometers constructed and repaired | 62        |
|    |  | 7         |

4 752

<sup>1</sup> Only 10 malaria units were in operation up to June 30 1946

set c- . . . . . he hope that the Philippine  
Gov s yearly appropriations for  
mal the activities being under  
taken by the units of the United States Public Health Service

TABLE 3—*Expenditures and encumbrances of the Malaria Control Division broken down by items from Jan 1, 1946 through Mar 31 1948*

| Items | Jan 1 1946 to<br>June 30 1946 | July 1 1946, to<br>June 30 1947 | July 1 1947 to<br>Mar 31 1948 | Total        |
|-------|-------------------------------|---------------------------------|-------------------------------|--------------|
|       | \$63,837.11                   | \$233,757.01                    | \$712,701.31                  | \$510,295.43 |
|       | 2,857.12                      | 8,813.03                        | 9,630.27                      | 20,900.39    |
|       | 1,674.34                      | 4,756.78                        | 4,343.90                      | 11,823.06    |
|       | 160.56                        | 650.30                          | 1,001.56                      | 1,812.42     |
|       | 3.75                          | 1,716.44                        | 882.32                        | 2,602.51     |
|       | 42.21                         | 500.73                          | 313.05                        | 857.99       |
|       | 2,200.00                      | 4,617.00                        | 17,734.89                     | 24,551.89    |
|       | 15,709.98                     | 73.94 10                        | 49,346.35                     | 134,071.42   |
|       | 49,692.16                     | 21,533.76                       | 7,005.65                      | 77,231.57    |
| Total | 134,879.27                    | 350,374.21                      | 304,023.25                    | 789,281.73   |

### ACKNOWLEDGMENT

The author is greatly indebted to Assistant Surgeon General Howard F. Smith, Chief of the United States Public Health Service in the Philippines and technical adviser to the President of the Philippines on public health and quarantine matters, for his valuable advice and encouragement and for his permission to use data contained in his

the manuscript.

cerning the work in other communities prevented their inclusion in this analysis. In these barrios, the campaign consisted of (1) treatment of cases with quinaquine hydrochloride, (2) vector control by means of larviciding with DDT and by use of automatic siphons and dams with sluice gates, and (3) education of the masses on simple prevention and control measures.

Permanent control devices such as automatic siphons, dams with sluice gates, and subsoil drainage are constructed whenever possible, and temporary measures like larviciding, brushing, and clearing are done only when permanent devices are impractical or cannot be immediately installed. Airplane spraying of DDT thermal aerosol<sup>2</sup>

spraying of dwelling places with DDT, benzene hexachloride, and chlordane to determine the effectiveness of this procedure, which has gained popularity in the United States and other countries. In the Philippines, however, the vector is not known to rest in houses at day time, and rural houses, which are generally far apart, are chiefly made of nipa and bamboo. A number of problems will have to be solved before residual spraying of houses can be adopted as a standard procedure in the Philippines.

With the concept that research work is indispensable for proper

country. From these studies was evolved the use of DDT treated sand<sup>3</sup> for the control of anopheline larvae in streams, which has been found to be highly effective, economical, and fast and which does not require sprayers or other apparatus. Studies are also being conducted with regard to the presence of vectors other than *Anopheles minimus flavirostris*, untoward effects due to atabrin, efficiency of new antimalarials, and improvement of automatic siphons. The Malaria Control Division has a small research laboratory in Manila where animal experimentation and preliminary testing of equipment

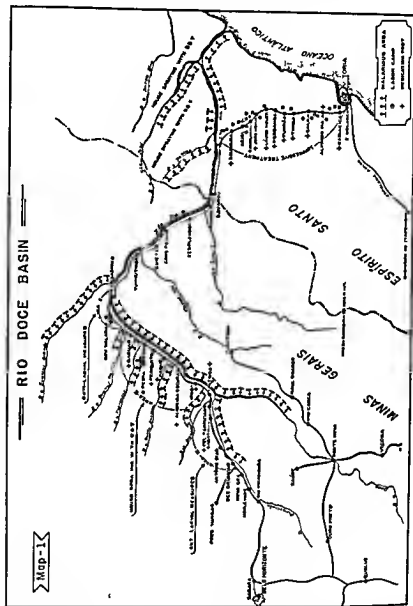
March 31, 1948, is \$789,281.73. Since the program is supposed to terminate on June 30, 1948, and even the over- . . .

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# MALARIA IN THE RIO DOCE BASIN, BRAZIL

H M PENIDO, M D, M P H, Director, Rio Doce Program, Serviço Especial de Saúde Pública, a cooperative organization maintained jointly by the Brazilian and the United States Governments

## INTRODUCTION

The Rio Doce Basin, which is located in the central part of eastern Brazil between the 38° and 41° S latitude and 40° and 44° of longitude west of Greenwich, has an area of about 90,000 square kilometers, part in the state of Minas Gerais, where the Rio Doce begins, and part in the state of Espírito Santo, where the river empties into the Atlantic Ocean, after travelling about 600 kilometers (map 1). The population of the whole basin, according to the 1940 census, was over 1,200,000.

The climate is quite regular, the major part of the basin being within the isotherms of 21° to 24° C. The lowest temperature recorded since 1943 in the malarious part of the valley was 10° C.

The pluviometric records of 20 years show a rainfall of 1,250 to 1,500 millimeters per year, badly distributed, falling generally between November and April and being followed by a dry period from May to October.

## THE MALARIA PROBLEM

In 1943, at the beginning of the sanitation program for the Rio Doce Valley, the only library reference encountered with respect to malaria in this region was a paper by Peryassu (1) dated 1922.

Therefore, an initial study was necessary in order to become acquainted with the essential facts regarding the transmission and distribution of malaria in the valley and principally along the Vitória Minas Railroad.

During 1943 Amaral and Penido (2) studied the distribution of *Anopheles* along the railroad and determined *Anopheles* (*A*) *darlingi* as the principal vector of the region. Near Vitória a domestic variety *A* (*A*) *albifarsus* was found, with an oocystic index of 13 percent. Later, with other investigations and with information from other areas, it was possible to obtain a better idea of the distribution of malaria in the entire basin, which showed a discontinuity of malarious areas not only in the Rio Doce but also in some of its tributaries.

1) With the exception of the area near the mouth of the Rio Doce, besides *A* (*A*) *darlingi*, *A* (*A*) *oreocoides* and *A* (*A*) *albifarsus* are the only vectors in the basin.

2) Malaria is transmitted throughout the year but with much greater intensity during the months of March, April, and May, at the end of

## CONTROL METHODS

## ANTILARVAL

Antilarval measures consisting of treatment of *A. darlingi* breeding places with paris green and occasionally ditching or filling were employed in the two ends of the malaria area, which begins near the city of Governador Valadares and extends to the station of Desembargador Drumond (areas I and III on map 1)

Area I is bordered on the north and east by the highly malarious valley of the Suassui Grande River, on the south by a range of mountains with the Rio Bahia road passing through the only valley, and on the west by the valleys of the Rio Doce and Suassui Pequeno Rivers, which are also malarious. In this area of approximately 500 sq km

relation among these zones and with the neighboring malarial areas.

Zone 2, more protected than the others being farther from the infested areas, has never been reinfested after the elimination of *A. darlingi* in 1944

Zone 1, separated from the valley of the Suassui Grande River by not very high mountains, was reinfested after 2 years. After eliminating *A. darlingi* a second time, this zone has continued negative until the present time (more than 1½ years)

The third zone, in direct contact with the malarial area of the Rio

same breeding place, a small lake situated between the roadbed of the railroad and the Rio Doce River. The other 16 breeding places of *A. darlingi* identified in this zone have been negative since September 1945, for 2½ years. This was accomplished in spite of the proximity of an infested area and a good means of transportation, such as the railroad

The same antilarval measures were carried out in area III (area II of map 2), which covers all of the malarious area of the Piracicaba River

In this area, the possibilities of reinfestation are smaller, for it may occur only from the infested area of the Rio Doce, the rest of the Piracicaba River Basin being well protected from other malarious areas by high mountains

The total number of *A. darlingi* breeding places found in this area covering 800 square kilometers, was 78, distributed along the Piracicaba River and 2 smaller branches, the Arrudas and Timoteo Rivers

Malaria is caused by 3 species of parasites. The cumulative data for 3 years (1944-46) show that in area II (map 1) out of 9,845 positive smears, collected from patients who came to the medication posts for treatment, 5,735 were *P. falciparum* infections, 4,025 *P. vivax*, and 86 *P. malariae*.

Malignant tertian infections are more frequent during the first half of the year when transmission is at its height. From June to December, most of the cases are due to *P. vivax*, probably because of relapses, which are more frequent in this type of infection.

The quartan cases are always few in number, occurring throughout the whole year.

### MALARIA CONTROL PROBLEM

In view of the extent of the malarial areas and considering that the greatest economic development was and will continue to be in those areas near the railroad, the control measures were carried out initially in this part of the basin.

Even with this restriction, two important facts had to be taken into consideration when planning for a control program for the area: first, the dispersion of the population, which for its greater part lives on farms, there being very few villages, generally about the railroad stations; second, the economic possibility of carrying out and maintaining control measures in such a large area.

Only a control method that would benefit the greatest possible number of people, cover a large part of the malarious area, and provide for a permanent solution of the problem would be suitable for this area.

Measures were taken into account against *A. darlingi* of the upper part of the Rio Doce Valley, along the railroad, between the towns of Governador Valadares and Desembargador Drumond (map 1). Besides this method, described in detail in an earlier publication (4), other control measures such as house spraying with pyrethrum and with DDT, treatment of patients with metoquin (atebrin), and with other antimalarial drugs, and suppressive treatment with metoquin, were employed in different parts of the malarious areas of the basin.

After nearly 5 years of work, we may evaluate the different control methods employed, according to their effectiveness in reducing malaria transmission and giving a definite solution to the malaria problem.

TABLE 1 Results of larval catches in areas I and III from August 1943 to March 1948

| Year | Quarter            | Area I |   |   | Area III |   |   |   |
|------|--------------------|--------|---|---|----------|---|---|---|
|      |                    | Zone   |   |   | Zone     |   |   |   |
|      |                    | 1      | 2 | 3 | 1        | 2 | 3 | 4 |
| 1943 | August<br>December | -      | + | + | +        | + | + | + |
| 1944 | 1st                | -      | + | + | +        | + | 0 | + |
|      | 2d                 | -      | + | + | +        | + | 0 | + |
|      | 3d                 | +      | 0 | + | +        | + | + | 0 |
|      | 4th                | 0      | 0 | + | +        | 0 | + | 0 |
| 1945 | 1st                | 0      | 0 | 0 | +        | 0 | 0 | 0 |
|      | 2d                 | 0      | 0 | + | +        | + | + | 0 |
|      | 3d                 | 0      | 0 | + | +        | + | 0 | 0 |
|      | 4th                | 0      | 0 | 0 | +        | 0 | 0 | 0 |
| 1946 | 1st                | 0      | 0 | 0 | +        | + | 0 | 0 |
|      | 2d                 | 0      | 0 | + | +        | + | 0 | 0 |
|      | 3d                 | +      | 0 | + | 0        | 0 | 0 | 0 |
|      | 4th                | 0      | 0 | 0 | +        | + | 0 | 0 |
| 1947 | 1st                | 0      | 0 | 0 | 0        | 0 | 0 | 0 |
|      | 2d                 | 0      | 0 | + | +        | 0 | 0 | + |
|      | 3d                 | 0      | 0 | + | +        | 0 | 0 | 0 |
|      | 4th                | -      | - | - | -        | - | - | - |
| 1948 | January            | 0      | 0 | 0 | +        | 0 | 0 | 0 |
|      | February           | 0      | 0 | 0 | +        | 0 | 0 | 0 |

- No larval catches

0 Larval catches but no *A. darlingi* identified+ Larval catches and *A. darlingi* identified.

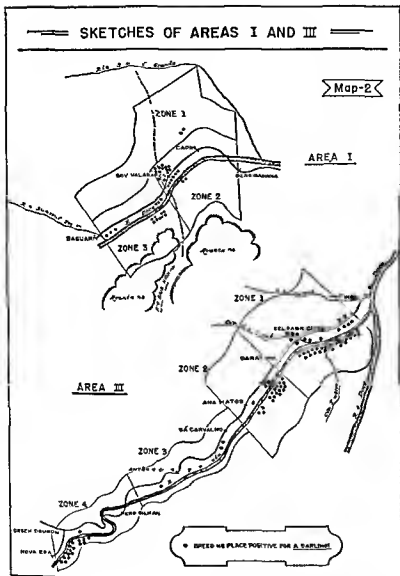
starting from such a small number. In May, another larva of *A. darlingi* was identified in the area, but after this finding, although the larvae catches continued intensively, no other larva of this species was found up to February 1948, and no malaria cases occurred.

Zone 3 never showed favorable conditions for the development of *A. darlingi*. Only three breeding places of this anopheline were found. The area, which was cleaned up by the second quarter of 1945, remained thus up to February 1948.

Zone 2, much closer to the reinfestation area, offers good conditions for *A. darlingi* breeding. The total number of breeding places in the area was 21. It was cleaned out by December 1946 and remained negative for *A. darlingi* larvae throughout 1947 and the first 2 months of 1948.

Zone 1, close to the contaminated area, shows the same problem as zone 3 of area I. Of the 38 *A. darlingi* breeding places found, only 1 is continuously reinfested, the other 37 having been negative for more than a year. Several houses of this zone were sprayed with DDT in September 1946 and in January and October 1947.

The results obtained with the application of antilarval measures in these two areas may be considered satisfactory as far as immediate results are concerned, for malaria transmission was completely discontinued.



Zone 4, the farthest from the reinfestation point, with 16 *A. darlingi* breeding places, was cleaned up by April 1944. Reinfestation occurred after 3 years in April 1947, when 1 larva of *A. darlingi* was found. It was then decided to make no attempt to clean the area again, in order to see if this anopheline could maintain itself here,

ures, house spraying with DDT will be the method chosen for malaria control in rural areas, where the population is scattered. Aside from simplicity of execution and excellence of immediate results, this method is practical.

#### MALARIA TREATMENT

In April 1947, an experiment was started with some of the new antimalarial compounds, in order to verify the possibility of obtaining a clinical cure with the administration of a single dose. Camoquin (SN 10,751), chloroquin (SN 7,618), oxo chloroquin (SN 8,137-5) and paludrin (SN 4,888) were distributed, and a follow up of all treated cases was established to verify the therapeutic value of the drugs as well as the appearance of toxic effects (7). The maximum single dose was 10 grain to adults over 14 years of age. Up to February 1948, 677 cases were treated with camoquin, 42 with paludrin, 19 with oxo chloroquin, and 18 with chloroquin.

The results to date are encouraging, and it is felt that it will be possible to obtain at least a clinical cure after a single dose of camoquin. We have not had enough experience with the other drugs, but chloroquin and oxo chloroquin seem to produce the same effects.

#### DISCUSSION AND CONCLUSIONS

Of the three types of control measures employed, treatment was the least valuable. Although further experiments with the new antimalarial drugs may show better results than those obtained with metoquin, it is difficult to realize an effective control method for rural areas, based solely on medication.

Closer to the desired aim come the antimosquito measures, because of their ability to reduce or discontinue malaria transmission.

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But when a definite solution for the malaria problem is taken into consideration and the eradication of the anopheline vector is a possibility, the choice of control measures will probably be a combination of antilarval and antiadult measures.

Up to the present, we have obtained encouraging results with the application of only antilarval measures. Failure, represented by reinfestation, does not seem to deny the possibility of achieving this aim.

Analysis of reinfestation in the two clean areas where antilarval measures were applied shows that it was not so frequent as would be expected if proximity of infested areas is considered. When rein

## V MALARIA

As a permanent method of control, the results were not so because reinfestation occurred. But, if we consider the fact that the area is cleaned, the expenses of policing the *A. darlingi* breeding places are small, and that if reinfestation occurs it can be easily checked, this control method can be considered satisfactory for permanent measure.

### ANTI ADULT MEASURES

In 1943, weekly and biweekly house sprayings with pyrethrum were employed experimentally in two small villages of area II (map 1) Baguari and Pedra Corrida, in an attempt to control the malaria epidemic occurring at that time.

No appreciable results were obtained, and an explanation of this fact came later with the progress of the studies on *A. darlingi* biology (3), most of the insects leaving the houses during the day which showed that this anopheline has well-defined nocturnal habits.

Later on, in September 1946, house spraying with DDT was employed as a control method in area II and was followed by a second cycle in 1947, the interval between the two cycles being 4 months. The immediate results were very good, for the expected yearly epidemic of 1947 was brought to a minimum (5).

To determine whether a control method based on the killing of adults would eliminate from an area a species of anopheline considered of high domesticity and low density (6), an experiment was started in part of area II, a week before the house spraying with DDT was begun.

Around the village of Naque, in the central part of area II, a weekly cycle of larval catches was established covering an area of 70 square kilometers. The first DDT spraying cycle was started a week later, October 1946, and was repeated in February 1947, covering all houses in the area. A DDT wettable powder was applied in a concentration of 25 grams of DDT per square meter of internal walls in the first cycle and 20 grams per square meter in the second cycle. Fifty three breeding places were searched at weekly intervals for 53 weeks. The total number of larvae collected was 26,148, of which 1,298 were identified as *A. darlingi*.

DDT spraying did not seem to affect the larval production of *A. darlingi*. In fact, the same pattern of growth in the number of larvae was observed as in years before, when no DDT was employed. This number, which was at a low level in September 1946, increased progressively until June 1947, and then decreased continuously to the end of September 1947, last week of observation. The fact that 33 of the 53 breeding places were dry by the end of the dry season on the breeding of mosquitoes is well illustrated. Nevertheless, it is possible that a decisive result may be obtained after a few more cycles of DDT residual sprayings have been made. Because of its economic advantages over antilarval meas-



## INTRADERMAL TEST IN MALARIA

JACK G. MAKARI, B.A., M.D., D.T.M. AND H. (Eng.), Senior Physician, Trans Arabian Pipe Line Hospital, Beirut, Lebanon

The present methods available for the diagnosis of malaria especially in its chronic and latent forms, are not satisfactory. The need, therefore, for a specific test to detect those persons whose tissues have been sensitized to the malarial parasites is great. The difficulty

species specific, but that the results were uncertain

infected with *P. knowlesi* give positive complement fixation with sera of humans infected with *P. vivax* or *P. falciparum*. Furthermore Kligler and Yoeli (5) showed that *P. gallinaceum* antigen gave positive complement fixation test with sera from human cases of malaria which reacted with *P. knowlesi* antigen. The latter result suggested the desirability of using *P. gallinaceum* as the source of antigen for an intradermal test in human malaria.

### METHODS

*Preparation of antigen*—The antigen was obtained from the blood of a chicken heavily infected with *P. gallinaceum* (90 percent of cells infected). Heart blood was put in small oxalated tubes in 2 cubic centimeter amounts. Plasma was separated by centrifugalization and discarded. The corpuscles were washed with saline four times, following which they were desiccated in vacuo. After a period of 2

in a mortar. A suspension containing 1 percent of this powder in 0.5 percent carbolyzed salt solution was then prepared. This suspension was incubated for 24 hours at 37° C. It was then passed through a Seitz filter and tested for sterility. This sterile fluid was employed in the skin tests.

In performing the skin testing 0.1 millilitre of the stock extract was injected intradermally into the skin of the forearm. Readings were taken at 24 hours. The diameter of redness was recorded in millimetres. The following scheme was used in recording

festation did occur, it was promptly checked. Furthermore, it was

With the continuation of control measures, it will be possible to

units consisting of its tributaries and to study the possibility of eradicating *A. darlingi* in each of these smaller areas. A certain amount of the yearly budget for malaria control should be employed in cleaning up one or more of these smaller areas, while temporary control measures are continued in other parts. In this way it will be possible to obtain definite results in controlling the disease in many of the malarious areas of the basin.

Assuming that eradication is only partially obtained and that infested areas will continue to represent a menace to clean areas, the

Brazil, an effort should be made to study the possibility of eradication of this anopheline from certain of these areas, for a situation similar to the one encountered in the Rio Doce Basin may occur.

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series showed a negative cephalin cholesterol flocculation test, the percentage of positive cephalin reactors being 15

*Group 2, cases with malarial history within 3 years*—Of the 81 cases of chronic malaria followed up in 18 months, 75 (92 percent) developed a positive intradermal test (5 mm and above). In this same series during the first interview, 69 (85 percent) had positive cephalin flocculation tests, 61 (76 percent) had detectable splenic enlargement, and only 54 (67 percent) had positive blood films (fig 1, b)

*Group 3, cases where malarial history goes beyond 3 years*—In some of these cases, the malarial history goes back as far as 20 years (fig 1, c)

*Results*—Nineteen of the twenty one cases studied had a positive intradermal test (90 percent) varying between 1+ and 2+. The cephalin cholesterol flocculation test done on these same cases was positive in only one case (5 percent), being 1+

### DISCUSSION

This study indicated that the *P. gallinaceum* antigen gives positive intradermal reactions in human malaria. It is positive not only in the acute and chronic cases but also in the latent cases, and in people whose infection dates back 20 years. In all the cases studied, the 24 hour reading gave the best results, the reaction becoming much less noticeable in 48 hours. In only three cases in the whole series was there an immediate reaction in the form of an urticarial wheal.

There was no focal or general reaction in any of the cases tested except in one patient who had had the last attack 8 months prior to the testing. A few hours following the intradermal injection the patient had chilliness which lasted for 2 hours and which was followed by a

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 4+ at 2 to 5  
 months after the last attack. At 1 year it tends to come down to 2+,  
 after which it is maintained at this level or comes down to 1+ but  
 With 1+  
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Diameter of redness

|                  |     |     |     |     | Reading |
|------------------|-----|-----|-----|-----|---------|
| 0-4 mm           | --- | --- | --- | --- | 0       |
| 5-9 mm           | --- | --- | --- | --- | +       |
| 10-14 mm         | --- | --- | --- | --- | ++      |
| 15-19 mm         | --- | --- | --- | --- | +++     |
| 20 mm. and above | --- | --- | --- | --- | ++++    |

## CHOICE OF CASES

Cases studied consisted of three groups

*Group 1, cases with a negative malarial history (controls)*—This

*Results*—Of the 70 cases tested 5 had a 1+ reaction 1 a 2+ reaction and 63 had a negative reaction the percentage of positive intradermal tests being 7.7 (fig 1 a) The volunteer showing a 2+ reaction comes

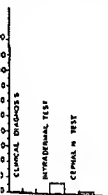


Figure 1 a

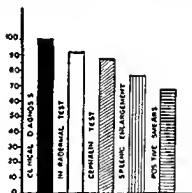


Figure 1 b

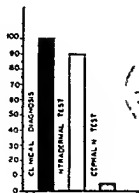
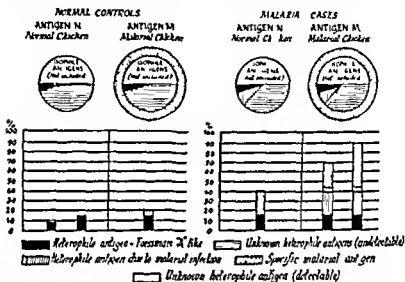


Figure 1 c



92 percent positive reactors were detected. When this same group was re tested 8 months later, 71 percent positive reactors were found. However, pseudopositive reactions never exceed 1 to 2+, and of the 11 pseudopositive reactions obtained with malarial antigen, 8 also gave reactions of similar intensity with normal antigen. It is thus evident that running control tests with the normal antigen is of value in eliminating about 57 percent of pseudopositive reactions when malarial antigen is used for the test.



**SCHEMA.**—Schematic representation of normal and malarial antigens "N" and "M" their antigenic components, and the part each plays when injected intradermally in normal and malarial individuals. Height of blocks represents percentage of positive

intradermal reactions. The two columns in the normal control section using antigen "N" represent two values obtained with two different areas. Those in the malarial group section, antigen "M" represent values obtained for the same series at 8 months' interval.

Figure 2 Results of intradermal tests

The nature of these false positive reactions raises a point of academic interest. Why should normal chicken antigen give weak positive results when given intradermally to normal individuals? Why should it give even high percentages in chronic malaria cases? Hyde (6)

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there is a relationship between the Forssman hapten and the specific substance of group A. It was thus found desirable to investigate any existing relation between pseudopositive reactions and the blood groups of the individuals giving these reactions in the control series.

infection is about the same. In the distribution of these positive reactors, there is a shift from 2 to 4+ in group 2 to 1 to 2+ in group 3. The cephalin test, however, drops markedly from 87 percent in group 2 to 5 percent in group 3 (fig 1).

The correlation coefficient ( $r$ ) of the intradermal test with other variables in group 2, reveals some interesting features (8). There is a very close relationship between the intradermal test and the activity of the malarial infection as shown by the cephalin cholesterol flocculation test,  $r$  being +0.37. The correlation between the intradermal test and the last attack is a negative one, i. e., the nearer the last attack, the stronger the test. The correlation between the intradermal test and hepatic enlargement is higher than that between the test and splenic enlargement, whether at the time of the testing or during the acute phase.

### CONTROLS WITH NORMAL ERYTHROCYTE ANTIGEN

Eaton and Coggeshall (4) working on complement fixation in human malaria found that an antigen prepared from normal monkey erythrocytes fixed complement with some sera from malarial patients, al

M prepared from a malarial chicken with 90 percent infection of its red cells, as described before.

### METHODS

Antigen N = 14 = 2 = 12 = 1 = 2 = 2 = 1 = 1 = 1 = 1

years of age where a negative malarial history and a negative splenic enlargement were found. These were street boys to be taken to a summer camp. Their poor economic and nutritional status corresponded well with that of the Armenian refugees at Anjar used as the malarial group.

### RESULTS

The results of the intradermal tests on normal controls, using 14 percent pseudo malarial antigen the normal reactors. The results on malarial cases showed 40 percent positive reactors to the normal chicken antigen. When malarial antigen was used on malarial cases,

be of any great moment from the point of view of the public health, each false diagnosis of syphilis may have a serious and even disastrous implication for the person concerned. This is particularly true of chronic or latent malaria where failure to demonstrate malarial parasites leads to positive serum reactions being falsely attributed to syphilitic infection. The author thinks it would be safe to reckon on the false positive serum reaction due to malaria disappearing within a month of the start of antimalarial treatment if this is by the 'long course' method, lasting 6 to 8 weeks. The intradermal test is of great help in pointing out false positive reactions due to malaria.

*Problem 3 concerns malaria in the blood plasma program*

In such a program, it is essential that malaria be ruled out before a donor is accepted. Woolsey (11) as early as 1910 had reported the development of clinical manifestations of malaria in a 59 year old male following a transfusion from a malarial donor. Since then a large number of cases have been reported. In 1938 Wright (12) reviewed the literature on the subject, collected 23 cases, and reported 6 additional ones.

It is evident that examination of a blood smear cannot be considered an absolute safeguard, nor is the history. This is emphasized by Gordon (13), who suggests the advisability of rejecting as donors those born in or coming from a country where malaria is endemic. With the introduction of large masses of nonimmune population into the United States during wartime, Gordon's suggestion that young men have to be sent back to their homes, and old people.

By the use of the intradermal test, it is possible to rule out those who have had malarial infection. Those with a positive intradermal test should be disqualified as blood donors regardless of negative history and negative smear findings, since the infection may be latent for years and still be transmissible.

*Problem 4 concerns the problems created by returning malaria carriers*

Freeborn (14) emphasized the importance of these problems and pointed out that the United States have as many as 3 of the malarial parasites among foreign origin.

Very large numbers of carriers may be expected when troops return from the fighting fronts. It would be quite impracticable to keep such troops under surveillance sufficiently long to ensure that they are free from infection before returning to their homes.

43 percent belonged to group A

With reference to the second question, Eaton and Coggeshall (4) showed that malarial infection stimulates the production of other hetero antibodies, which react with normal monkey erythrocytes, lack

The production of these hetero antibodies in malaria may explain why normal antigen gives even higher results in chronic malaria cases

We would ascribe the 71 to 92 percent positive reactions obtained with the malaria antigen in chronic malaria cases to the rise of specific immune bodies, the presence of which has been established beyond doubt by Taliaferro, Eaton, and others.

#### APPLICATION OF THE TEST

So far I have referred to the use of the intradermal test in detecting those individuals whose tissues have been sensitized to the malarial proteins through a malarial infection, present or past (8) In another work (9) I described the use of the cephalin cholesterol flocculation test as a good index of the activity of the malarial parasites in the tissues of the host

A combined use of these two tests would help to solve many problems in the field of malariology A few of those problems are reviewed with suggestions which might help toward their solution

*Problem 1* is the detection of chronic, masked, and latent forms of malaria, and the determination of the activity of the infection

The intradermal test described herein is not only positive in malaria cases suffering from the disease at present but also in those with a positive malarial history even though the last attack may have been 20 years before It could thus be used to detect all forms of malarial infection in its chronic, masked, or latent forms for diagnostic purposes

To differentiate active from latent type of malarial infection the cephalin cholesterol flocculation test is resorted to, it being positive in the active cases

*Problem 2* is malaria as a cause of false positive serological reactions.

Dawber (10) in a recent paper stresses the importance to the individual of a false positive diagnosis of syphilis, saying that although false positive serological reactions may be so infrequent as not to





As a solution to the problem, Dr L. L. Williams, Jr, proposed eradicating malaria from the United States by antianopheline units to control the expected explosive epidemic outside such areas. Such an antianopheline programme, aside from being very costly, is very difficult if not impossible to accomplish. For the whole mosquito population has to be annihilated from the States before that end could be achieved. The best solution for such a problem seems to be twofold:

(1) By a thorough case finding project of malaria carriers among the troops before returning to their homes. Such necessitates the use of the intradermal test on all the members who have served in malarial districts. Those with a positive intradermal test should have their blood examined with the cephalin cholesterol flocculation test. The positive cephalin reactors with active malaria should be given thorough treatment. In this way the chances of these individuals

in the country and  
the disease is endemic  
here when done on

and the problems created by returning malaria carriers minimized

### SUMMARY

(1) An intradermal test with an antigen obtained from *P. gallinaceum* was done on 81 proved active malaria cases. Of these, 75 (i. e., 92 percent) gave a positive test, as compared with 67 percent positive smears, and 87 percent positive cephalin tests.

(2) The test when done on 21 cases with a positive malarial history that goes beyond three years showed a positive reaction in 19 (i. e., in 90 percent) varying between 1+ and 3+. The cephalin test done on the same cases showed only one weak positive reactor (i. e., in 5 percent).

(3) The test was negative in 63 of the 69 cases with a negative malarial history.

(4) Preparation of the testing solution and the methods used in reading and interpreting results are also given.

(5) The mechanism underlying the test is thought to be based on hypersensitivity of malarial sensitized tissues to a common antigenic factor in *P. gallinaceum* of chickens.

(6) A control antigen prepared from normal chicken erythrocytes was also used. This was positive in 14 percent, while malarial antigen was positive in 20 percent of the control group.

(7) Pseudopositive reactions were all weak and did not exceed one plus. Of these pseudopositive reactors, 90 percent were of group O.



## Session 5 MALARIA CONTROL

Saturday, May 15—9 30 a m to 12:00 m  
Departmental Auditorium, Main Hall

### THE MALARIA CONTROL PROGRAM OF THE UNITED STATES ARMY DURING WORLD WAR II

JAMES STEPHENS SIMMONS, M D, PH D, DR PH, Sc D (hon)  
Brigadier General, U S Army (Retired), Dean, Harvard School  
Public Health, Senior Consultant in Preventive Medicine to the  
Surgeon General, U S Army, and the Secretary of War

#### INTRODUCTION

Malaria has been recognized for centuries as a major hazard of armies operating in tropical and subtropical countries. This fact was reemphasized by the experience of the allied forces in World War II which showed that malaria still plays an important role in human affairs.

The history of this allied experience is an interesting story of a great cooperative effort to protect the largest aggregation of fighting men ever mobilized for service in the malarious regions of the world.

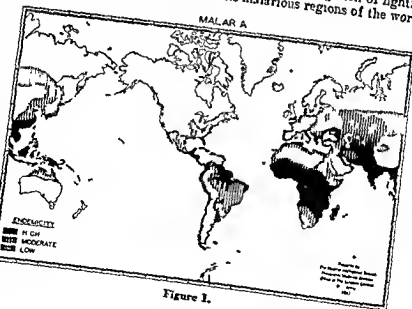


Figure 1.

It deals with timely advances in our fundamental knowledge of malaria and the development of useful new agents and methods with which to control the disease; it also deals with a few tragic and costly

malaria would require a consideration of the important work of the United States Navy, the Public Health Service, and many other American agencies; and also the contributions of our allies—particularly the British—who did so much to advance the common cause. How-

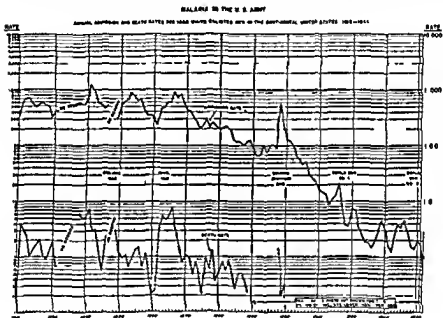


Figure 2.

When this country began to prepare for World War II, medical officers of the Armed Forces had a long background of experience with malaria extending back for more than a century and a half. This had

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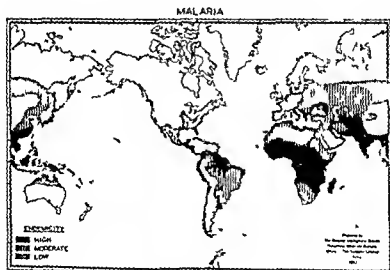


Figure 1

on Tropical Medicine of the Army Epidemiological Board and later, of the Board for the Coordination of Malaria Studies

Special emphasis was placed on the following activities (1) Collection of information about the distribution of malaria and its mosquito vectors in all regions to which Allied troops might be sent, (2) training of military personnel, both medical and line, in malaria

available information to the practical problems of the war. The collection of information was carried out by the Medical Intelligence Division which prepared surveys indicating the malaria hazards of all overseas theaters and recommended the precautions to be taken by the commanders of all troops sent abroad.

The training program included (1) Stimulation of teaching of tropical medicine in civilian medical schools, (2) establishment of special postgraduate courses in tropical medicine at the Army Medical School, Tulane University, and elsewhere, (3) provision for training in malaria control for medical personnel at the Army Field Medical

civilian scientists. It was coordinated with the programs of the Navy and the Public Health Service and was spearheaded by the joint medical research program sponsored by the National Research Council and operated by the Committee on Medical Research of the Office of Scientific Research and Development. This great national research effort together with our basic knowledge of malaria (And

An important contribution was the development of specific information about the suppressive use of atabrin which showed its superiority to quinine and proved that when given in doses of 0.1 gm daily atabrin prevents *P. falciparum* malaria and suppresses infection with *P. vivax* (Shinnon, J. A., et al, 1944, Farley, N. H., 1945). Field application of this information made it possible for our troops to function in spite of infection. Later, other useful drugs were discovered, including chloroquine which, when administered, were used

reduced and brought under fairly satisfactory control in the peacetime army. Thus in 1939 our troops were living in well sanitized garrisons, and they were rarely exposed to infection except during occasional field maneuvers in endemic areas. In that year the hospital admission rate for malaria in the total prewar army was only 4.9 per thousand per annum.

It was realized, however, that this excellent record could not be maintained if the country became engaged in a tropical war. For years our troops had contracted malaria during maneuvers in the Philippines and Panama, and on at least one occasion in Panama in 1935 field maneuvers were actually abandoned because of the high infection rate. The speaker (Simmons 1938) commented on this incident as follows: "Such occurrences show the importance of malaria

warning of the dangerous situation that would undoubtedly arise should it become necessary for our army to operate for a long period in the American Tropics." Thus when war finally came, officers of the Medical Department were not only aware of the military importance of malaria, but they realized that we were poorly prepared to meet the disease in the field.

#### DEVELOPMENT OF WARTIME CONTROL PROGRAM

Because of the military importance of our defenses in the Caribbean and the Pacific, special emphasis was placed on plans for prevention of the tropical diseases, particularly malaria.

Active planning for the wartime malaria control program began early in 1940. The objectives were (1) to expand immediately the mosquito control and other facilities required to protect troops training in the southern part of the United States, and (2) to develop more effective methods for their subsequent protection under combat conditions abroad. In the Surgeon General's Office, the program was developed with the assistance of a group of experts assigned to the Preventive Medicine Service. These included Col. Karl R. Lundeborg, William S. Stone, William Hardenburgh, and later, Paul F. Russell, Oliver McCoy, Justin Andrews, and others, most of whom subsequently served in overseas theaters of war where they assisted in guid-



on Tropical Medicine of the Army Epidemiological Board and later, of the Board for the Coordination of Malaria Studies

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extensive  
try and

civilian scientists. It was coordinated with the programs of the Navy and the Public Health Service and was spearheaded by the joint medical research program sponsored by the National Research Council and operated by the Committee on Medical Research of the Office of Scientific Research and Development. This great national research effort together with the parallel British program, added much to our basic knowledge of the epidemiology, treatment, and prevention of malaria. (Andrus et al, 1948, Tidy and Kutschbach, 1948)

An important contribution was the development of specific information about the suppressive use of atabrin which showed its superiority to quinine and proved that when given in doses of 0.1 gm daily atabrin prevents *P. falciparum* malaria and suppresses infection with *P. vivax* (Shannon, J. A., et al, 1944, Fairly, N. H., 1945). Field application of this information made it possible for our troops to function in spite of infection. Later, other useful drugs were discovered, including chloroquine which, when administered only once a week, suppresses *vivax* malaria and cures *falciparum* infections in 1 to 2 days. Also, useful new antimosquito agents were developed including the Army insect repellent, the aerosol insecticide spray bomb, and later, various preparations of DDT which were used extensively for killing adult and larval mosquitoes.

One of the most important features of the program was the development of plans for a special malaria control organization designed to survey, plan, and execute all the procedures necessary to protect troops in the field (Russell, 1943). This organization included medical officers, units head engineers

men. The personnel were trained in various places with the assistance of the Tennessee Valley Authority, the Rockefeller Foundation, the Florida State Board of Health, the Pan American Highway Commission, and later, at the Army School of Malariology in Panama. Such malaria organizations were assigned to all tropical theaters of operation to serve under the theater surgeon. Their function was to plan, supervise, and help carry out measures for malaria control, to provide technical advice to unit commanders and assist them in developing malaria discipline among their troops, also to advise concerning the filling, draining, and spraying operations to be done by the engineer troops and by native labor.

The importance of these malaria survey and control units to the efficiency of our forces cannot be overemphasized, for they contributed much to the successful termination of the war. Russell (1946) commented on their value as follows: "Allied malaria control units have demonstrated the value of malaria control by modern methods all over the world with such striking success that civilian authorities are more willing than ever before to budget funds for antimalarial programs. Already there are plans in hand for extensive work in such widely separated areas as the Southern United States, Brazil, West Africa, Italy, India, and Australia, and in each case based to a considerable degree on lessons of World War II."

Still another important feature of the Army's malaria control program was the effective quarantine set up to prevent the introduction of the disease or its vectors into this or other allied countries.

In addition to these preventive activities, the Surgeon General developed in the Division of Medicine under Brig Gen Hugh Morgan and Col Francis R. Dieuaide, a highly efficient and successful program for the treatment and hospital care of all soldiers who contracted malaria.

The measures employed in the control program in the outline on page 833 published by Russell in 1943 separate the measures applicable to fixed installations from those suitable for field operations.

#### RESULTS OF CONTROL PROGRAM

As expected, malaria was the most important disease faced by American troops. A total of 460,800 United States soldiers were admitted to hospitals for malaria, a rate per annum of 189 per thousand. This figure does not represent the actual infection rate,

on Tropical Medicine of the Army Epidemiological Board and later of the Board for the Coordination of Malaria Studies

Special emphasis was placed on the following activities (1) Collection of information about the distribution of malaria and its mosquito vectors in all regions to which Allied troops might be sent (2) training of military personnel both medical and line in malaria

collection of information was carried out by the Medical Intelligence Division which prepared surveys indicating the malaria hazards of all overseas theaters and recommended the precautions to be taken by the commanders of all troops sent abroad

The training program included (1) Stimulation of teaching of tropical medicine in civilian medical schools, (2) establishment of

School and special courses for malariologists provided in Panama and elsewhere and (4) training in malaria control for all line officers and troops These courses were supplemented by a campaign of health

and the Public Health Service and was spearheaded by the joint medical research program sponsored by the National Research Council and operated by the Committee on Medical Research of the Office of Scientific Research and Development This great national research effort together with the basic knowledge of malaria

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however, for many of the admissions included relapses, and undoubtedly many infections occurred which were suppressed or cured by the routine field use of atabrin. About 80 percent of the clinical cases were admitted to hospitals overseas, and the admissions in this country were largely for patients with relapses from infections contracted abroad. The patients received excellent clinical treatment and medical care, and the death rate was insignificant. According to Dieuaide (1945) the actual illness usually lasted only 3 days, the average stay in hospital was about 7 days, and only 7 percent of the men had to be evacuated to the United States.



Figure 3.—Rates for 1944 are provisional, based on weekly statistical reports, and exclude cases resulting from overseas exposure. Malaria admissions per thousand men per year in Army in the continental United States: World War I—World War II (includes only infections presumably acquired in the United States).

#### THE AMERICAN THEATER

Malaria control was more effective in the American theater than in the other malarious war zones because military conditions made it

United  
by the  
control measures, which

recorded in the Army, being  
1944, and only 0.1 per thousand in 1945 (fig 3). There were only

about 4 000 cases during the entire war. The joint programs of the Army, Navy, and Public Health Service were also of great benefit to the civil population.

#### CLASSIFICATION OF MEASURES OF MILITARY MALARIA CONTROL<sup>1</sup>

A Measures applicable to fixed installations (including permanent and semipermanent posts, camps, fields, and stations in the United States and overseas)

##### 1 Environmental measures

###### (a) Protection against adult mosquitoes

###### (b)

- (1) Draining
- (2) Filling
- (3) Use of larvicides.
- (4) Miscellaneous

##### 2 Individual measures

- (a) Curative treatment
- (b) Use of sleeping nets (mosquito bars)
- (c) Use of repellents
- (d) Wearing of protective clothing
- (e) Malaria instruction and discipline

#### B Measures applicable to field operations

##### 1 Individual measures

- (a) Use of sleeping nets (mosquito bars)
- (b) Use of repellents
- (c) Wearing of protective clothing
- (d) Prophylactic treatment
- (e) Malaria instruction and discipline

##### 2 Environmental measures

- (a) Protection against adult mosquitoes
  - (1) Spray killing with pyrethrum extract.
  - (2) Selection of suitable camp sites
  - (3) Residual spray DDT in houses, etc.
- (b) Control of mosquito larvae whenever feasible

work, and of the extensive civilian programs conducted by various countries of the Western Hemisphere with the collaboration of the

<sup>1</sup> Modified from Russell (1943)

Pan American Sanitary Bureau, The Rockefeller Foundation, and the Institute of Inter American Affairs, represented a vast contribution to the health of civilians in this hemisphere

#### PACIFIC THEATERS

The Army's experience with malaria in the islands of the Southwest Pacific was tragic, and the disease interfered seriously with military progress. As shown in figure 5, the admission rates were extremely high in 1942 and 1943. Several divisions, both Marine and Army,

#### ADMISSION RATE FOR MALARIA U S ARMY BY MONTH 1942-1947

##### LATIN AMERICAN AREA

RATES EXPRESSED AS CASES PER 1000 MEN'S HEADS PER YEAR

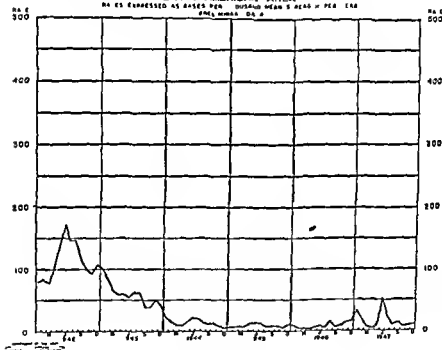


Figure 4

were immobilized for months, between October 1942 and April 1943, one third of the Army admissions to hospital in the Southwest Pacific area were caused by malaria. Harper (1946) estimated that 100,000 Allied troops were infected in the South Pacific area alone, and, since each man averaged 2 attacks, the total loss was many millions of

The joint programs of the  
were also of great benefit

#### CLASSIFICATION OF MEASURES OF MILITARY MALARIA CONTROL <sup>1</sup>

**A Measures applicable to fixed installations (including permanent and semipermanent posts, camps, fields, and stations in the United States and overseas)**

##### 1 Environmental measures

- (a) Protection against adult mosquitoes
  - (1) Selection of suitable camp sites
  - (2) Screening of buildings
  - (3) Spray killing with pyrethrum extract.
- (b) Control of mosquito larvae
  - (1) Draining
  - (2) Filling
  - (3) Use of larvicides
  - (4) Miscellaneous

##### 2 Individual measures

- (a) Curative treatment.
- (b) Use of sleeping nets (mosquito bars)
- (c) Use of repellents
- (d) Wearing of protective clothing
- (e) Malaria instruction and discipline

#### **B Measures applicable to field operations**

##### 1 Individual measures

- (a) Use of sleeping nets (mosquito bars)
- (b) Use of repellents
- (c) Wearing of protective clothing
- (d) Prophylactic treatment
- (e) Malaria instruction and discipline

##### 2 Environmental measures

- (a) Pre-
  - (1)
  - (2)
  - (3)
- (b) Control of mosquito larvae whenever feasible

Effective programs were also carried out in the Canal Zone, Puerto Rico, and the Philippines.

<sup>1</sup> Modified from Russell (1943)



As the troops moved north for the liberation of the Philippines and the attack on Japan, the field malaria control program became more effective. Malaria discipline and the suppressive use of atabrin were better enforced, and the control units developed many new methods for

on the fighting strength in certain areas in the Philippines, especially on Luzon where the disease had practically reached epidemic proportions among the disorganized civilians. For example, the admission rates for clinical malaria in the Sixth Army were less than 40 per 1,000 on Leyte compared with a rate of 100 on Luzon. The admission rates for all United States troops in the Pacific theater during the war are shown in figure 5.

#### THE MEDITERRANEAN THEATER

The malaria control problems were also difficult in North Africa, Italy, and the islands of the Mediterranean. The admission rates among our troops were relatively high during the early part of the invasion, but the malaria control organization began operations at a fairly early period, and an effective program was developed by Col Justin Andrews, the chief malarologist, under Col William S. Stone, who was chief of the Preventive Medicine Service for our forces. According to Colonel Andrews (1946), "The admission rates were higher in 1944 (61 per 1,000) but it is believed that a greater proportion of troops were infected in 1943, due to poor malaria discipline, imperfect atabrin supply, and inadequate antimalaria organization. With the correction of these defects, the rates for primary malaria became less in 1944 and 1945."

He stated that the most important vector of malaria was *Anopheles labranchiae labranchiae*. Principal gametocyte reservoirs were rural Arab populations and Italian prisoners of war in North Africa, civilian refugees, Italian prisoners of war, impressed Yugoslav laborers and Italian cobelligerent troops in the remainder of the theater.

The special antimalaria organization finally developed was strongly centralized. The theater malarologist commanded a detachment of malarologist officers. These were attached to major commands in which they gave technical direction to malaria survey and control detachments. An airplane dusting and spraying flight detachment

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were not applied vigorously until crippling epidemics made prompt action imperative

New Guinea and Guadalcanal will be remembered in history as examples of military inadequacy comparable with Pearl Harbor. Fortunately, this situation was soon corrected, and commanders who were previously unimpressed with the importance of malaria were forced to take active steps to develop an aggressive campaign against the disease. The theater established priorities which enabled the War Department to supply the required malarialogists and trained malaria survey and control detachments and also to insure the shipment of

ADMISSION RATE FOR MALARIA U.S. ARMY BY MONTH 1942-1947  
SOUTH PACIFIC AND SOUTHWEST PACIFIC AREAS

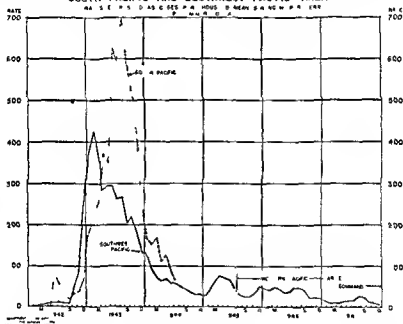


Figure 5

necessary control supplies. The Army and Navy cooperated with our allies in the development of area wide joint operations for the

- (4) centralized control of policy and personnel coupled with decentralization of operation, (5) integration of survey and control activities, and (6) an effective training and education program

## CONCLUSION

In conclusion it may be stated that the wartime experience of the United States Army with malaria was a trying one. The malaria control program conducted in camps in the United States was successful, and the admission rates for troops infected in this country were lower than at any time since the Revolutionary War. However, as was expected, the rates in overseas tropical theaters were high, especially during early combat periods before extensive environmental control could be applied.

## ADMISSION RATE FOR MALARIA US ARMY BY MONTH, 1942-1947

## CHINA, BURMA AND INDIA

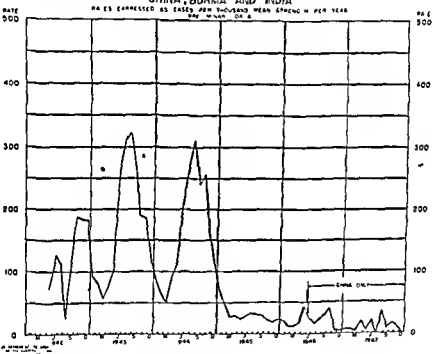


Figure 7.

By February 1946 the malaria admission rates for the United States Army had decreased to less than 10 per thousand in all overseas theaters except in the Western Pacific and Asiatic areas. It is impossible to determine the influence of suppressive atabrin on these

out the theater and their use stimulated by special training and subsequent reminders. In 1943 all troops were ordered to take suppressive atabrin. That policy was liberalized in 1944 by exempting troops in areas where the malaria hazard was negligible. During 1945 suppressive atabrin was directed only for troops in areas where malaria was an uncontrolled danger. During 1944 the value of residual spraying with DDT was demonstrated for the first time—in this theater.

Malaria control in the Mediterranean theater was highly effective, and the control organization and procedures developed by Colonel

#### ADMISSION RATE FOR MALARIA U.S. ARMY BY MONTH 1942-1947 MEDITERRANEAN AREA

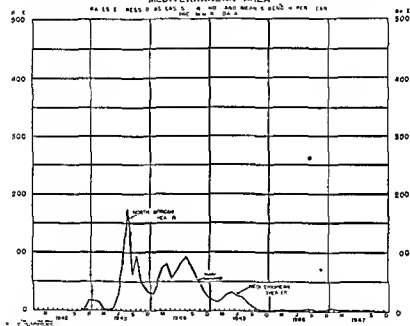


Figure 6

Andrews afford a pattern for use in the civilian control of malaria. The results are shown in figure 6.

#### CHINA BURMA INDIA THEATER

The experience in the China Burma India theater also showed the difficulties of malaria control in the field and emphasized the effectiveness of malaria survey and control organizations. It is regretted that there is not time in this talk to discuss the details of results obtained in this theater. The admission rates are shown in figure 7.

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The routine use of atabrin for the prevention of *P. falciparum* infection and the suppression of *P. vivax* was of great value in many

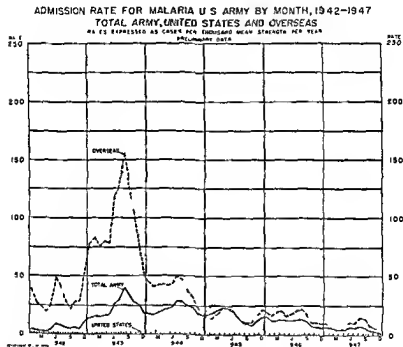


Figure 8.

The research program was of enormous benefit in the war and for the future. We have come out of this bitter wartime experience with much new knowledge about malaria and its vectors. We now have better drugs for treatment and suppression of the disease. We also have better methods for repelling and destroying mosquitoes which can be applied effectively and economically for the control of malaria among civilians in many tropical countries. However, we still need a true prophylactic suitable for military use in the field, and it is important that research be continued in the attempt to find better methods for the protection of troops against malaria.

ion facilities, electricity, etc. It is axiomatic, that as our town is being built, national unsanitated villages immediately spring up in the adjoining area. This is done largely for economic reasons, as most of the male population in such groups hope sooner or later to become employees of industry and to enjoy the financial as well as the social prestige which are far beyond what they have been accustomed to. Their small houses are without water and as a rule have no lights, nor are they provided with screens, and in many instances no latrines are constructed. The houses are generally built from mud reinforced with twigs and branches or with palm or bamboo. Such areas are actually seedbeds for the spread of many types of infection and because of proximity are a source of danger to the population in the unsanitated areas of industrial villages nearby. We have no control over such villages, therefore a malaria campaign confined to our towns alone is relatively non-effective since we are separated only by a wire fence or arbitrary boundary.

Contract was presented which was mutually satisfactory to the Ministry of Public Health and the Creole management. We undertook to underwrite the program for a definite period in our area of highest endemicity, after which time both parties agreed to renegotiate the financial burden.

Studies, both clinical and entomological, and costs during the first contract.

The first area chosen was the company and national towns of Arapito and their environs, which is a malarialogist's dream, these wamps, and squatters were identified in eastern Venezuela. The problem therefore became interesting to a number of specialists. Partly due to this program I was requested to establish an office in Caracas to assist in the able to secure a highly qualified sanitary engineer, doctor of this office. A senior or provisional sanitary engineer was assigned to the field with appropriate

# APPLICABILITY OF TECHNIQUES OF MILITARY MALARIA CONTROL TO INDUSTRIAL DEVELOPMENTS AND RURAL POPULATIONS

A. GAGE, M. D., *Medical Director, Standard Oil Co (New Jersey)*  
*Caracas, Venezuela*

Malaria with its world wide distribution and high morbidity and mortality rates is one of the most serious medical and economic problems with which we are confronted. It is no respecter of youth, age, race, or sex. It is a disease that its control is a difficult task, and its cure, and

in human cases, we cannot with absolute certainty predict a cure by the use of the most modern remedies. This is substantiated by numerous relapses among large groups, especially when vivax infections predominate. We know very little of the mechanism of spontaneous cure and practically nothing of the extraordinary phase in the human being.

As the literature is flooded with articles on these subjects, it would perhaps be of more interest to cite the mechanics of a specific program, which is illustrative.

For this purpose, an oil concession in eastern Venezuela has been chosen. This is operated by the Creole Petroleum Corp., an affiliate

of the Standard Oil Co. In the tropics, the inexperience, tact, and medical program to such programs to a degree is able to the stock

holders for financial outlay versus returns on investment. These procedures often consume much time and effort, therefore, the presentation of such recommendations must be concise, objectives clearly portrayed, and expected end results explained. Business, like the

is aware of this fact, and more stress is being laid on preventive medicine in its broad aspects and to a less extent on the curative program, although we must still maintain modern hospitals, clinics, and field units for the sick.

In eastern Venezuela with its jungles, plains, and swamps, the control of malaria has always been a serious problem. For some reason, known only to the geologists, oil is seldom found near modern cities, therefore we choose a town site in the bush and build a modern town provided with water and sewage systems, stores, schools, recrea-



clustering in the large field areas. We finally constructed a truck provided with an air compressor and the necessary agitators to mix the solutions or emulsions, depending upon the need. Individual portable tanks were filled at the truck, and by use of the compressor about 40 to 60 pounds of air were introduced into these tanks, which were carried by individuals of the squad, one man per tank. Special nozzles

ere used when necessary.

A survey was made of the total number of houses in this original area and an estimate made of the total surface area to be sprayed, as we intended using approximately 200 milligrams of DDT per square foot. This area contained a minimum of about 12,772 inhabitants, living in 2,861 houses of various descriptions. The surface area in individual houses varied from 190 to 432 square meters. The estimated total area was 769,704 square meters. The cost to cover this area was estimated at 37,419.00 Bs. (At present exchange, 3.33 Bs. equals 1).

On all smooth surfaces, such as plaster walls with hard surfaces, we used 5 percent solution of DDT in kerosene, while an aqueous suspension was used on adobe walls and palm thatched houses.

The work was so planned that the operation would be continuous,

from such work reported by others.

It was our intention to continue and intensify our statistical data both from the clinical and entomological standpoints. Due to certain losses of personnel, very little entomological work was done this year, of course, exceedingly unfortunate. We have, however, acquired sufficient personnel to resume our studies on the adult

mosquito campaign, since the time was far too short to draw definite conclusions. Seasonal or annual variations, cyclic periods, control of breeding areas, and suppressive medication must be included, and several years may be necessary before we can definitely

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Standard light traps and portable traps large enough for animal bait, were constructed

Adult mosquitoes in the latter were removed at 2 hour intervals from dusk to sunrise in order to determine the periods during which the various anophelines were prone to feed. Counts were made of the total catch which was then separated into anophelines and others

sionally reached a peak of 1 000 per trap per night. Unfortunately all this material from June 1945 to December 1947 and certain figures

had been placed in the States for large amounts of DDT, certain equipment and for the necessary solvents before the end of the war, therefore, when this material was officially released we had sufficient data on which to base preliminary work of actual house spraying

that proved successful in the actual spraying of the houses and premises within the area mentioned

It was not until the early part of 1947 that work actually began in this joint program between the Creole Petroleum Corporation under the general supervision of the Creole Medical Director of the Eastern Division and the Director of the Division of Malariaology of the Venezuelan Government. We reviewed the literature of such operations and endeavored to adapt our equipment insofar as possible to that used by the Army. However, certain modifications were necessary, and it was deemed inadvisable to use airplanes for spraying or

ness of the people to take it once per week compared to their active antagonism in taking a drug four to six times per week as was necessary with atabrin or quinine. Our suppressive treatment had been used for many years previous to the war.

Such parties are also provided with the most modern insect repellents, one of which is made especially for us. Its effect has been gratifying, and reports from the field show it is efficient for ap-

... for a certain number of sprays within or without the tents or trailers which may be in use.

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which they are working or in which they intend to work at a later date. Here again preliminary surveys are undertaken by our Caracas group, and various phases of general sanitation, public health measures, and entomological studies are coordinated for the benefit of such groups.

It is our feeling that definite progress has been made in this anti-malaria campaign using techniques developed during the war and certain preventive techniques which we had developed over a period of years prior to the last conflict.

### SUMMARY

An attempt has been made to illustrate the methods by which measures for malaria control based on Army techniques can be applied to industry.

Antimosquito and malaria campaigns were undertaken by the Venezuelan Government and Creole Petroleum Corporation in eastern Venezuela the early part of 1947 using certain techniques which were developed by the United States armed forces during the late war. The objectives were (a) to reduce the incidence of malaria in a specific

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uplicated in certain other of the Jersey affiliates in malarial zones.

### ADDENDA

listed would be more or less around the circumferences

Table 1, summary of costs for spray program

Table 2, consumption of certain antimalaria drugs for the first quarter of 1947 before our campaign compared with the same

fluctuates of course with salaries and types of occupation. If our campaign has reduced malaria by only 10 percent, it is evident that the financial outlay is highly justified. From our statistics, we had 362 malaria cases in eastern Venezuela during the month of August

of this spraying were demonstrated visually. In fact almost too much good will was produced, for in certain other areas which in our opinion did not need this type of campaign, the people themselves clamored

it articles the editors apparently gave free rein to their imaginations, little realizing, we hope, the effect upon masses of population, particularly throughout Latin America. For instance, an apparatus which produces a smoke type of fog under rather high temperature

effect until they began finding dead insects scattered along the floors for a period of months.

So far our management is convinced of the necessity for continuing this work, and we hope to intensify our efforts in the original areas as well as in certain others which are being surveyed at the moment.

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Wangtop) and feels that we are making better progress than formerly, not only because of the efficiency of the drug itself but also the willing

TABLE 2 — *Antimalarial drug consumption (eastern division)*

| Drug    | First quarter<br>1947 | First quarter<br>1948 |
|---------|-----------------------|-----------------------|
| tab. in | 24,100                | 2,500                 |
|         | 348                   | 24                    |
|         | 232                   | 32                    |
|         | 8,700                 | 750                   |
|         | 5,150                 | 500                   |

time factor in 1948. We do not believe that this marked reduction is due solely to the campaign, however, it has no doubt played a very definite part. The use of Aralen, better control of drugs, and intensified work in the field must be considered. The figures are at least interesting.

### Bibliography

#### DDT UNIT COSTS FOR CARIPITO

DDT (100 percent) is indicated as used only partially in Caripito.

There is a gap in the work from May 22-29, inclusive, but the payroll shows these days. Therefore this labor cost has been excluded in computing unit costs but has been included in total and for over all cost.

SAS shows daily labor costs of Bs. 189 for same periods that Creole shows Bs. 176. Creole figures are used.

Kerosene estimated at 20 l per kilogram of DDT.

#### Material costs

DDT (100 percent) = Bs. 5.83/kg — Caripito

Deenol (50 percent DDT) = Bs. 5.38/kg — Caripito

Kerosene = Bs. 0.1/1 — Caripito

|   |              |
|---|--------------|
| Truck costs (operation depreciation maintenance)                              | = Bs. 78/day |
| Depreciation of special truck equipment + pumps (over 2 years)                | = Bs. 5/day  |
| Equipment total   | = Bs. 83/day |
| Based upon original cost of uniforms etc. and estimated life of 1 year — cost | = Bs. 10/day |
| Add Creole supervision at   | = Bs. 20/day |
| Other costs — total   | = Bs. 30/day |

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<sup>1</sup> Due to the fact that this paper has been written on generalities rather than the intricate details of such a program, we wish to acknowledge the great assistance afforded by the above authors as individual references were not given in the body of this paper.

TABLE 1.—Caripito summary of costs of DDT residual spray program

| Cycle No. | Location                             | Dates (1947) |         | Num-ber of days | Num-ber of inhab-itants | Num-ber of houses | Houses per day | Aver-ages M <sup>2</sup> per house | Total M <sup>2</sup> treated | Total DDT used <sup>1</sup> | Total Ker (lb) used | Materials |       | Total costs <sup>2</sup> |       |            |             |            |
|-----------|--------------------------------------|--------------|---------|-----------------|-------------------------|-------------------|----------------|------------------------------------|------------------------------|-----------------------------|---------------------|-----------|-------|--------------------------|-------|------------|-------------|------------|
|           |                                      | From         | To      |                 |                         |                   |                |                                    |                              |                             |                     | DDT       | Ker   | Mat-erial                | Labor | Equip-ment | Other costs | Total cost |
| 1         | Parcela y Talencia                   | April 8      | April 8 | 2               | 428                     | 110               | 55             | 180                                | 20,020                       | 84                          |                     | 8         | 474   | 285                      | 566   | 60         | 987         | 8.88       |
| 1         | El Rincón                            | 10           | 14      | 5               | 308                     | 195               | 35+            | 308                                | 54,000                       | 238                         |                     | 1         | 200   | 718                      | 418   | 130        | 2,543       | 14.49      |
| 1         | La Manera                            | 18           | 17      | 5               | 1,295                   | 377               | 102            | 169                                | 57,174                       | 218                         |                     | 1         | 1,172 | 490                      | 248   | 160        | 2,081       | 6.82       |
| 1         | La Esparza                           | 18           | 24      | 9               | 2,002                   | 284               | 42+            | 266                                | 802,212                      | 441                         |                     | 2         | 2,378 | 1,520                    | 747   | 270        | 4,912       | 12.78      |
| 1         | Los Norcos                           | 20           | 25      | 1               | 318                     | 79                | 79             | 143                                | 11,750                       | 48                          |                     | 2         | 258   | 176                      | 83    | 30         | 647         | 6.93       |
| 1         | Carritillos Chorritos y Morillos     | Apr 29       | May 8   | 7               | 1,775                   | 378               | 84             | 775                                | 160,863                      | 428                         | 304                 | 2         | 2,319 | 1,231                    | 581   | 210        | 4,337       | 11.46      |
| 1         | Caripito                             | May 6        | May 10  | 14              | 2,001                   | 698               | 42+            | 314                                | 187,600                      | 721                         |                     | 2         | 848   | 2,482                    | 1,122 | 420        | 8,089       | 13.48      |
| 1         | Los Muelles, Las Bochas y Las Palmas | 20           | 20      | 1               | 131                     | 42                | 43             | 158                                | 8,675                        | 35                          |                     |           | 188   | 159                      | 83    | 90         | 478         | 11.11      |
| 1         | km 14                                | 21           | 21      | 1               | 240                     | 71                | 71             | 197                                | 14,000                       | 56                          |                     |           | 302   | 176                      | 83    | 30         | 891         | 8.32       |
| 1         | El Pavozito (camp)                   | 30           | 10-6    | 42              | 2,268                   | 476               | 20+            | 232                                | 110,253                      | 319.3                       | 1,000               |           | 1,000 | 2,110                    | 906   | 360        | 5,630       | 11.09      |
| 1         | La Floresta (camp)                   | June 11      | June 20 | 10              | 521                     | 181               | 18+            | 451                                | 78,320                       | 157.6                       | 8,168               |           | 920   | 1,780                    | 850   | 360        | 4,125       | 22.80      |
| 1         | Hospital Turm Ref (camp)             | 23           | 25      | 5               | 48                      | 60                |                |                                    | 21,075                       | 42.2                        | 809                 |           | 248   | 980                      | 415   | 150        | 1,778       |            |
|           | Totals and averages                  |              |         | 70              | 12,775                  | 2,893             |                | —                                  |                              |                             |                     |           |       |                          |       |            | 76,081      |            |
|           | Labor (unaccounted)                  |              |         | 8               |                         |                   |                |                                    |                              |                             |                     |           |       | 1,409                    |       |            | 37,410      | 12.72      |

<sup>1</sup> In bellows (B).<sup>2</sup> DDT marked (\*) 50 percent wettable unmarked

## SPECIES SANITATION AS APPLIED TO THE ERADICATION OF (A) AN INVADING OR (B) AN INDIGENOUS SPECIES

FRED L. SOPER, *Director, Pan American Sanitary Bureau,  
Washington, D C*

The term "mosquito eradication" has been widely misused in the literature where mosquito reduction is meant.

Species sanitation may be defined as the reduction of the density of a given species below its effective transmission threshold through selective measures adapted to its biology.

Species eradication may be defined as the ultimate in species reduction and implies the world wide extermination of a species. Such eradication has been recorded for the passenger pigeon and the dodo, but no instance is known in which a mosquito species has been exterminated by human agencies. The term has been applied, rather, to the elimination of a given species in a limited, though at times extensive, area. When such limited species eradication has been accomplished, the species may reappear after the interruption of control measures, if, and only if, it be reintroduced. The threat of reintroduction varies with the size of the cleaned area and with its isolation from infested territory. Such terms as local, national, regional, and continental species eradication are useful.

In 1941 a paper was presented (Soper and Wilson, 1942) entitled, "Species Eradication: A Practical Goal of Species Reduction in the Control of Mosquito-borne Disease." This paper was based on intensive campaigns against individual species resulting in the eradication of *Aedes (Stegomyia) aegypti* in certain parts of Brazil and in Bolivia and of *Anopheles (Myzomyia) gambiae* from northeast Brazil without the elimination of other local species of mosquitoes.

Both *Aedes aegypti* and *Anopheles gambiae* are African in origin and elsewhere must be considered as invading species. The ability of these species to invade floral regions other than that of their origin is based on the adaptation of their aquatic phases to universally found foci, namely, artificial receptacles for water in the case of *aegypti*, and shallow sunlit pools without vegetation for *gambiae*.

In Africa, *Aedes aegypti* occurs both as a domestic and as a sylvan species. In the Americas, where it was introduced several centuries ago, it is very much at home as a domestic species but has failed to

Africa is both a forest and a  
re it survived for a decade and  
were reached, it never came to

the forested regions and was combatted only as an essentially domestic species.

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collaboration of the authorities of Paraguay, Brazil, Uruguay, and Argentina, and (2) in the northern part of the continent, with Venezuela, Colombia, Ecuador, and the Guianas collaborating. With the great central part of the continent covered by campaigns already in existence in Brazil, Bolivia, and Peru, the success of work in these two regions will mean a continent free of *aegypti*.

The campaign against *Anopheles pseudopunctipennis* in the Pacific slope valleys of South America is feasible because of the peculiar geographical conditions which make piecemeal eradication possible. These valleys are short and narrow, isolated from the rest of the world by the high Andes to the east and the Pacific Ocean to the west and from each other by long stretches of absolute desert. Those charged with carrying out the project in Peru demonstrated to their satisfaction, on the basis of apparent local eradication, that *Anopheles pseudopunctipennis* could be eliminated, but did not carry the project to completion because of administrative and personnel difficulties (Rockefeller Foundation Annual Report 1944). In Chile, *pseudopunctipennis* can no longer be found in the valleys of Tarapacá Province, and malaria has been absent since 1945.

The finding of *Anopheles gambiae* at Wadi Halfa in the Sudan below the Second Cataract, in May 1941 (Lewis 1942), was followed in March 1942 by a sharp epidemic of malaria in southern Egypt among the Nubian villages along the reservoir above the Aswan dam. A survey by S. Madwar showed that *gambiae* was already at Aswan and at many other points far below the dam. The news of this invasion and of the terrifying epidemic which struck the Nile Valley as far north as Asyut, only 200 miles from Cairo, in the fall of 1942, was suppressed by wartime censorship. The Egyptian Ministry of Health organized a special service in 1943 to protect the lower Nile Valley and the delta from invasion and to eradicate *gambiae* from the 550 miles of infested valley in Egypt. No further extension of *gambiae* downstream occurred, but the flood season of 1943 came before *gambiae* could be eradicated, and that year's autumn epidemic paralyzed the life of Upper Egypt. An official committee which investigated the situation early in 1944 estimated that 135,000 persons had perished in 1942 and 1943. Large landowners reported that the production of food crops had decreased by from one third to one half in the stricken areas. That the tragedy was not much greater was

or elsewhere.

In spite of these limitations on previous experience, the authors cited above boldly pointed out some attractive eradication possibilities for indigenous species of mosquitoes

(1) *Anopheles pseudopunctipennis* from the Pacific slope valleys of Peru, (2) *Aedes aegypti* from Egypt, (3) *Anopheles culicifacies* from Ceylon, and (4) *Vector anophelines* from islands in general. These selections were made because of definite limits placed to the problem in each case by geographical conditions and their influence in hampering reinfestation.

In the intervening years various workers have had further experience, not always successful with species eradication programs involving *Aedes aegypti* in South America. *Anopheles pseudopunctipennis* in Peru and Chile. *Anopheles gambiae* below the Second Cat

in the Mediterranean

Brazil south of Bahia were practically cleared of *aegypti* and the known infested area of northeast Brazil was shrinking. Much of Peru and British Guiana is now clean, and Bolivia has been free of *aegypti* for a number of years.

To protect their investment in species eradication and to maintain their local and national freedom from *Aedes aegypti*, Bolivia and

culties consequent to the war effort permitted only Peru, Bolivia, Brazil, and British Guiana.

Buenos Aires in September 1947. Brazil proposed that the Pan American Sanitary Bureau should coordinate the campaigns in different countries for the eradication of *aegypti* from the Americas. After approval by the Council, initial steps were taken for the organization of two regional campaigns: (1) in the River Plate, with the

of the terrain and the large size of the island (9500 square miles) No insoluble problems have been encountered, and ultimate success is anticipated

Evidence from British Guiana (Giglioli, personal communication) indicates that residual spraying with DDT will eradicate *darlings* in some areas

In analyzing the additional experience with eradication during recent years, it should be noted that the campaign against *Anopheles*

eradication of indigenous species, it is significant that many workers have become convinced, on the basis of field observations that eradication can be accomplished. The eradication of *S. and Sardinia* promise to throw their attempt to go beyond species of indigenous anophelines merits special attention

eradication campaign, whether against invading or indigenous species. When the invading species is actively spreading, the prevention of further expansion at the periphery must be the first consideration. In the antitambora campaign in Brazil great care was taken early to clean the peripheral zone and to protect an additional marginal zone against infestation, even at the expense of operations in the more central epidemic area. In the case of *Aedes aegypti*, which was more stabilized in its range, eradication was carried out as would have been done with an indigenous species, first in the large port cities with gradual extension to ever widening tributary areas.

Long term security demands that eradication campaigns include points where the species may not appear to be a menace. Thus, in the city of São Paulo, Brazil, where the degree of infestation was never high and local outbreaks of yellow fever were unknown, eradication of *aegypti* was obtained in order to prevent the city from continuing as a seedbed for the reinfestation of other parts of Brazil. In a similar way, the continental eradication program must eventually include several countries, including the United States, where the immediate danger from this species may be inapparent.

... for the large areas, ... om ... uch ... ier ... wise have been neglected. Hundreds of towns and villages in Brazil are today free of *aegypti* because it has been more economical to clean them than to maintain permanent staffs in the larger cities which they threatened.

ruary 19, 1945. All control measures were discontinued before the end of that year, and the species has not reappeared.

In the meantime, the Sudan Medical Service (Lewis 1944) organized an eradication service south of the Egyptian border and pushed *gambiae* once more above the Second Cataract of the Nile River.

The campaign which led to the eradication of *gambiae* in the

in the oases where *Anopheles sergenti* is found, malaria constitutes both a serious public health and an economic problem since the most important money crop, rice, is prohibited in the areas close to the villages. Early in 1946 an *Anopheles* eradication campaign was undertaken in the Kharga and Dakhla Oases lying far out in the desert. In this campaign DDT was used as a larvicide throughout the widely scattered areas which comprise these two oases. The picture was deliberately complicated by canceling all restrictions on the grow-

thought that the finding of anophelines at that time was due to failure to eradicate and not due to reinfestation. However, it is now over a

cessful against the two most dangerous species, *Anopheles maculipennis clutus*, a swamp breeder, and *Anopheles superpictus*, a mountain stream breeder. The work during the first season was limited to the Karpas Peninsula and a small northeastern section of the island and was highly successful.

The 1947 program included the whole of the northern range of mountains and the main plains from the eastern to the western ends of the island.

and February 1948.

The campaign in Sardinia began after a careful survey in 1946. The program calls for the use of DDT as a residual house spray and as a larvicide. The principal difficulties encountered are the roughness

obtained with DDT and other residual insecticides in the one shot control of all household insects is bringing together once more the campaigns against yellow fever and malaria in general house disinfection services for the control of these and other diseases transmitted by domestic insects. Future eradication proposals must be

tend  
any of

spraying is required for other insects, special eradication campaigns against single species may be unnecessary

It must be admitted that eradication is not a universal panacea and should be advocated only for the solution of carefully selected problems. On the other hand, there is often a tendency to deny the feasibility of eradication because of the careful attention to administrative detail required. This tendency exists in spite of the work with *aegypti* and with *gambiae* and in spite of no less than 20 examples of eradication of introduced agricultural pests in the United States (Lyle 1947) since 18

(1923)  
counties

42), and the citrus blackfly in Florida (1934-35). On the other hand, the demonstration that eradication is possible with *Aedes aegypti* and *Anopheles gambiae* has caused some workers to fail to appreciate the difficulties inherent in the method and to display unwarranted enthusiasm for eradication campaigns against other disease bearing insects. A sane balance must be kept, and all factors bearing on each individual case must be examined. One would not recommend eradication of *Anopheles quadrimaculatus* in the United States, for example, since its northern range is so much more extensive than the present distribution of endemic malaria that the cost would be out of line with the benefits.

control is leading some public health workers to say that of eradicating certain noninsect transmitted communicable diseases which are still all too common by intensive application of known methods of control. The time is not far distant when the health worker will cease to glory in the reduced incidence of preventable disease but will rather be obliged to accept the full responsibility for such preventable disease as does occur in the population under his care.

Partial control services are an unending financial drain and are difficult to maintain permanently on an efficient basis. In the long run, eradication is less expensive, especially if the initial attack is pressed during that season of the year when conditions are least favorable for the breeding and propagation of the species.

Local eradication leads logically and irresistibly to a demand for regional and even continental eradication. The elimination of *gambiae* from Egypt and the Nile Valley below the Second Cataract in the Sudan should create a demand for the eradication of this species up stream for a thousand miles until regions are reached where *gambiae* is able to maintain itself as a forest mosquito. Then, and not till then, should the permanent barrier zone of protection against reinfestation be established. A careful study of the problem of *gambiae* transmitted malaria throughout the range of this mosquito should reveal a number of other regions where eradication may be feasible even though it be necessary to maintain a constant barrier against reinfestation. In the same way, eradication of *aegypti* is feasible for Egypt, for the Middle East, for other parts of the Medi-

species wherever possible

by Gorgas in 1901, and by Oswaldo Cruz at Rio de Janeiro, Brazil, beginning in 1903, were general campaigns directed against all mosquitoes. It is true that these early campaigns were expensive in men and money.

Gorgas:

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(1911) 1

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dangerous local anopheline. Thus species sanitation became the goal of the malarialogist and of the yellow fever worker, each of whom went his separate way. At the present time, the striking results

in dense jungle I think in Strong's report on Liberia the statement is made that there too *gambiae* or *funestus* was not found in the heavy forest

Now the point is this If *gambiae* does not breed in dense shade, it is possible to eliminate it very cheaply by growing hedges I put the question to a doctor who is doing tsetse fly control in West Africa in the Gold Coast colony—would these hedges produce tsetse—and he looked at them and studied them carefully and he said emphatically "no" So the point is whether in permanent control, having got rid of the *gambiae* once, the cheapest way might not be to grow a hedge I am thinking very carefully of what we are going to do

center That is a fine question but it is a very important question which faces every man who has to deal with malaria in a place that is not isolated or which cannot be isolated

I hope that Soper will give us the evidence of *gambiae* breeding in shade. It is a question of fact. We have got to get the facts first because, as you all know, the wise (1) observation of Mark Twain says "First get your facts and then you can twist them as you like"

Dr F L SORR (United States) The question of *gambiae* and its habits is one which was first called very forcibly to our attention by Dr Barber when he visited the infested area in Brazil in 1939 Dr Barber had worked with *gambiae* in West Africa, and when he came

conditions in which we worked in Brazil, neither he nor any of the rest of us were ever able to find *gambiae* resting out away from human habitations.

With regard to the facts which Sir Malcolm has asked for, I can say that I have never had occasion in Africa to make any searches for

Dr Hadow who was cutting out swamps in the forest—I won't call it heavy forest but it was very definitely forest—with large numbers of monkeys and some elephants It was an area which had been depopulated a good many years ago because of the problem of sleeping sickness So there was no human habitation, and no human population living in this district We went out into the forest He had previously told me that during the dry season *Anopheles gambiae* was the most common mosquito which they caught in the forest at all levels, from right at the ground level up to 60 or 80 feet in the air, because they had catching stations at various levels.

We were in the forest at midday and the eight black boys that Dr

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## ABSTRACT OF DISCUSSION

SIR MALCOLM WATSON (United Kingdom) I had no intention of speaking this morning but a point has been raised of extreme practical importance and I would like to say a word about it.

Dr Soper said in the course of his remarks that *Anopheles gambiae* was a jungle breeder in Africa Is that right? (Soper nodded in the affirmative from the floor) Well, when I visited the copper mines of Northern Rhodesia in 1930 I had no familiarity with the habits of either *A. funestus* or *A. gambiae* so I spent most of my time hunting in their breeding places *Gambiae* I found breeding exclusively in the sunlight And Mr Harrison, who had been working there, had practically cleared the mining area in a few months of *gambiae*

*Funestus* is another problem It was breeding in the great swamps with these tall leaves in it which gave partial shade I also found

through Kenya with C B Symes and ultimately we came to the northeast corner of Lake Victoria where there was a rainfall of about 70 inches, and I found beautiful vegetation and at last a piece of jungle that I called shade. We dived in and hunted and got some thing but it was not *gambiae*

Next when I was asked to help in the mines of West Africa, the first thing that I wanted to know was what was breeding in the jungle So the man that I sent out was given the job of finding out and I sent me the reports the plans, and hundreds of observations



## MAN MADE MALARIA

W V KINO, *Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, Orlando, Fla*

Various human activities are important factors in the intensification of malaria. The expression man made malaria brings to mind first of all an image of borrow pits, drainage systems, and other from careless engineering or ever, reminds one that much of the malaria referable to this heading,

carelessness, indifference, or ignorance, and (2) collections of water unavoidably associated with agricultural practices or other human requirements. The total damage to health from these causes, although undoubtedly lessening, is still enormous.

### AVOIDABLE COLLECTIONS OF WATER

rainfall, and density of population. The importance of any one kind of drainage depends on the presence of a vector species.

tion they become favorable breeding places for a number of malaria vectors.

1 + 1.

All too frequently culverts under the road embankments are not placed low enough to permit the water to run out. Agricultural drainage ditches and canals often present similar conditions. In addition, ponds are formed when natural drainage is obstructed by the roadway embankments or canal spoil banks through which openings are not

Haddow had went into the forest and after probably 20 minutes they came back with—I think it was five *Anopheles gambiae*, and they insisted that these were mosquitoes that were biting them at that time. So I said to Dr Haddow, "Well are they very common here? Do they generally bite at this time of the day?" He said, "Sometimes they bite at this time of the day but if it is just *gambiae* that you want, the boys can get plenty." So I asked him to send the boys back in and have each one catch one *gambiae*. And by going back just off of the road and into the forest and beating the leaves a bit, all of them were back with *gambiae* in about 5 minutes. Here we have a technical

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be worth using a measurement like that in future observations. But there is always the point, too, that *gambiae* in some places may have totally different habits in one part of a country from another. We know that in case of *barbirostris* and various other mosquitoes

species whose production in rice fields has been correlated with malaria transmission are *A. freeborni* Aitl en in California, *A. pseudopunctipennis* Theob in Mexico, *A. aquasalis* Curry in Brazil and Central America, *A. labranchiae atroparvus* van Thiel in Portugal and elsewhere in southern Europe, *A. sacharovi* Favr, *A. sergenti* Theob, *A. superpictus* Grassi, and *A. hyrcanus* Pallas in the Near and Middle East, *A. hyrcanus sinensis* Wied in China, *A. annularis* v d Wulp in India, and *A. aconitus* Donitz in southeastern Asia and the Netherlands East Indies.

In view of the widespread association of malaria with rice culture it is of interest to observe that a notable exception occurs in the Philippine Islands where the disease is of relatively minor importance in the large rice growing valleys of Luzon and other islands. In that country the chief malaria vectors are members of the *minimus* group which are stream breeders. Although their larvae do not occur in the rice fields they may be found in ditches and canals that supply the fields with water.

In Java a serious malaria problem is associated with brackish water fish ponds. The highly efficient vector *Anopheles sundaicus* Rod., develops prolifically in these ponds with the result that the coastal areas are the most malarious parts of the island. Again, the Philippines provide a contrast in that similar fish ponds there are not malarious. *A. sundaicus* is absent and the two anophelines that do breed in the fish ponds *A. indefinitus* Lndl and *A. litoralis* King, are evidently nonvectors. This further illustrates the point that the importance of any one type of breeding place may vary greatly in different regions.

Water impoundments of various kinds and sizes comprise a large class of potential malaria producers. In this class are reservoirs for drinking water, irrigation, hydroelectric power, and mill ponds. In the United States the best known example of this class is the extensive series of artificial lakes formed by dams constructed in the Tennessee River and its tributaries. They are multiple purpose impoundments designed for flood control, navigation and production of electric power. The main bodies of such lakes are not favorable for mosquito breeding but the shore lines are highly favorable in many places where the water spreads out over grassy flats or gentle slopes. Early in this century smaller impoundments constructed by private concerns were responsible for numerous outbreaks of malaria and the disease also increased after the first impoundments in the Tennessee River. As a consequence when the Tennessee Valley Authority was formed and plans were drawn for the complete development of this river system, provision was made for malaria control coordinated with the construction program. Intensive biological and hydrological research has resulted in the development of an efficient system for the management of impounded waters in holding *Anopheles* production below the danger point. Of special importance

provided. Ponding also occurs as a result of improper handling of irrigation water when the excess is allowed to accumulate in low, undrained spots.

Other excavations, such as stone quarries, sand pits, and phosphate pits, are malaria hazards. Pits formed in excavating clay for adobe houses or for pottery manufacture are of special importance as they are usually close to habitations.

The anophelines associated with the waters in this category are the more generalized breeders. Representatives of this type of mosquito,

aside from this, are comparatively nonselective in their breeding habits. *A. funestus* Giles and *A. gambiae* Giles of Africa, although more restricted in habits, are also adaptable to a wide variety of breeding places.

Water filled wheel ruts are favorable for the production of some species of *Anopheles*. This kind of pool was of special importance during the military campaign in the Southwest Pacific, where *A. farauti* Lav and *A. punctulatus* Donitz are the chief vectors. In both New Guinea and the Solomon Islands heavy vehicular traffic over wet ground caused innumerable ruts which rapidly became populated with anopheline larvae. Extraordinary numbers of larvae, especially of *A. punctulatus* in New Guinea, were found in small newly formed pools of this kind even when completely free of vegetation, which is usually associated with the development of other species. Pools formed in bomb craters, shelter pits, or fox holes contributed to the military problems there. In these areas, too, the clearing of the jungles, a natural practice to those trained in malaria control in other regions, resulted in increased breeding, since these particular species of *Anopheles* avoid dense shade.

#### UNAVOIDABLE COLLECTIONS OF WATER

In the second category of man made mosquito breeding places, the extensive areas involved in rice culture are probably by far the most important. These areas are in the most densely populated malarious regions around the world. Rice, an indispensable food for millions of people, unfortunately requires almost continuous flooding and is thus responsible for an untold amount of malaria infection. Most of the important anopheline vectors commonly breed in rice fields, and in many areas they are the principal source of production. All the generalized breeders previously mentioned are known to occur in dangerous numbers in rice fields. For example, in the southern United States some of the highest populations of *Anopheles quadrimaculatus* have been recorded in the rice growing sections of Arkansas. Other

on and water impoundments. Cooperation of farmers in modifying their irrigation practices has had a beneficial effect. Continued research, public education, and suitable legislation must form the basis for the final solution of these as of other phases of the problem.

#### ABSTRACT OF DISCUSSION

Dr L J CHWATT (Nigeria) I would like to mention that out-of-door *Anopheles gambiae* shelters have been found in 1930, I believe, by Blacklock, recently confirmed by Muirhead Thompson.

is the establishment of certain basic principles, one of which is known as building malaria control into the reservoirs, or the permanent elimination of potentially troublesome marginal areas by filling and deepening, or by diking and dewatering.

Of other *Anopheles* breeding places that result from necessary human activities, mention may be made of wells such as are found in the oases of North Africa, on which the population is dependent for water for irrigation and household use. Other shallow wells, or

eastern Brazil, where it became established. Discovery of this fact was a critical factor in the successful eradication of the species from that region.

In Iran, production of *Anopheles* is said to be heavy in the algal growths in filter tanks. Artificial water containers for household use have occasionally been reported as important producers of *Anopheles*.

*Anopheles bellator* D and K, a species that breeds solely in the water collected in the leaf axils of bromeliads, was found to be an important vector in Trinidad. There these epiphytic plants grow in profusion on immortal trees that are planted in the cocoa fields for shade. This is perhaps the most unusual example of the associa-

from the indigenous malaria infected population. During military campaigns this was accomplished by removing the local population from the selected camp sites. In areas where moderate numbers of *Anopheles* are present but where malaria transmission has been eliminated or held at a low level, an outbreak of the disease may occur when gametocyte carriers are transferred or returned to these places. This danger appeared imminent in the United States upon the return of soldiers from overseas at the end of World War II. Fortunately, little trouble actually resulted from this cause, because the potential danger was anticipated, and steps to minimize it were taken by health agencies.

It is not within the scope of this paper to consider the control of malaria under the various conditions referred to. In the past, temporary measures have constituted a large part of the control programs, but they are being reduced as more permanent measures are developed by malaria workers. Regulatory legislation has been effectively applied to prevent harmful engineering operations in road construc-



# MANAGEMENT OF WATER TO CONTROL ANOPHELINE MOSQUITO BREEDING

CALVIN C KIKER, *Senior Sanitary Engineer, Malaria Control Branch,  
Division of Health and Safety, Tennessee Valley Authority, Wilson  
Dam, Ala.*

## INTRODUCTION

This paper deals with water level management for control of anopheline mosquito breeding as it has been developed and used in the southeastern part of the United States on impounded water. However, the principles and practices set out herein should have effective

the following measures is employed. (1) Mechanical removal of larval habitat, (2) management of water to eliminate or reduce the larval habitat, (3) control of mosquito breeding directly through water level variation, and (4) application of larvicides and other measures. Items 2 and 3 are dealt with, though 1 and 4 are briefly discussed since they are frequently employed in conjunction with water level management

The development of water-level management practice for control of anopheline mosquito breeding on impoundages in the southeastern

1900, 1911, 1922, and 1930)

*Effects of water level management.*—Although the management of

in which larvae are stranded on the draw-down, thus exposing them to destruction on the shore line. The aquatic predators include the top feeding minnows and several species of aquatic insects. The term "water level fluctuation" is inadequate, since an important feature of present-day practice provides a period of relatively constant water level just in advance of the mosquito season in the interest of limiting marginal growth.

*Types of reservoirs.*—For the purpose of this discussion, artificial impoundages are divided into two types, namely, (1) storage reservoirs, and (2) main-river reservoirs. The principles of water-level management for mosquito control will apply to either with equal effect-



ness, but operational requirements, together with factors having bearing on the mosquito potential, are usually different.

*Storage reservoirs*—Generally, storage reservoirs are located in the more mountainous, hilly, or rolling terrain found at the headwaters of main rivers. The marginal areas are usually relatively precipitous, which topographic feature in itself minimizes the mosquito breeding potential. Water is stored during the rainy season and released later during the dry season. In the southeastern part of the United States, this schedule normally results in a rising water level in advance of the mosquito season and a falling one after the season begins. A progressive stranding of drift and siltage occurs when the water is being lowered, together with the exposure of shore line kept free of vegetation by previous inundation, which results in an environment unfavorable to mosquito production. This is the normal operation in the storage reservoirs where adequate reservoir clearing plus a limited annual shore line maintenance, has resulted in almost complete mosquito control. However, if filling is delayed, as occasionally occurs, and the water level is raised slowly into growing vegetation after the season begins, a serious mosquito breeding problem may develop. Larvicide is applied as an emergency measure in such situations on TVA reservoirs. The total volume of seasonal larvicide has not been large in the storage reservoirs as may be appreciated from the fact that only one DDT larvicide airplane is required to provide coverage for seven storage and the three upper main river reservoirs of the Tennessee River development. These emergencies are not considered serious since they are usually of short duration. Figure 1 shows marginal conditions in a storage reservoir following recession of the water level during the mosquito breeding season. It is evident from figure 1 that this operation results in a shore line having very limited potential for mosquito production.

*Main river reservoirs*—By "main river reservoirs" is meant those which have a limited normal water level variation of 3 to 6 feet. Figure 2 shows the typical flat topography of this type reservoir.

They are usually located on the main river, and on multipurpose developments, particularly where a chain of projects occurs, special attention should be given water level management in the design of gates and turbines. The combined discharge capacities should be ample to provide the water regulation desired. Normal summer elevations should be established as well as the lower limits of drawdown for flood control, navigation, etc. In long reservoirs the basic clearing lines would be established for normal summer flow maintenance after impoundage. Clearing is usually applied to the normal summer maximum water elevation with upward movement for the backwater curve in long reservoirs. A chain of reservoirs on a river offers a special problem of design and operation considering the fact that the water levels or discharges from



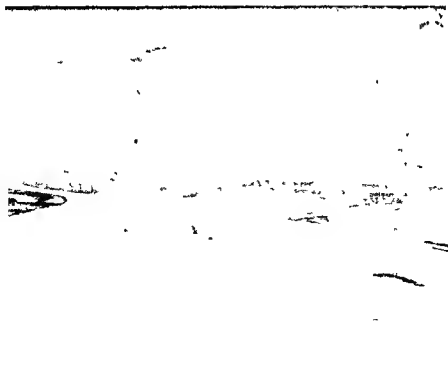
Figure 1







Figure 1



ing the water level above normal elevation and lowering it again, which strands drift and sludge, thus reducing the mosquito breeding potential along the margins. If no floods occur, the reservoirs are surcharged, anyway, in the interest of mosquito control.

(2) *Relatively constant level pool*—After the flood season or surcharging for the reservoirs, the water is maintained at normal elevation until the beginning of the mosquito breeding season, varying from about May 15 to June 15 in the region of the Tennessee River, which tends to limit the extent of marginal growth invasion. If flood control operations are not in progress or in prospect, the main stream reservoirs are filled to the normal summer maximum elevation by about April 1 to 15, which serves mosquito control interests reasonably well in marginal growth management. An earlier filling, say about March 15, would coincide better with the beginning of the growing season in the Tennessee River Valley, but, on an average, flood control operations are not over by this time.

(3) *Periodic water level fluctuation*—Where mosquito breeding has progressed to a significant point, periodic water level fluctuation of about 1 foot in scope at weekly intervals is initiated. This has not been difficult since some weekly variation in water releases normally occurs, which is regulated and accentuated in the interest of mosquito control. Fluctuation in one reservoir affects other reservoirs in the chain, and further variation in the size of a reservoir produces corresponding variations in the scope of periodic fluctuation for any given

of the Tennessee River, use of stored water is begun on normal operations about July 1 or midseason. This is in the interest of utilizing stored water for power development and to provide storage space for flood control during the succeeding winter and spring. The exact time of beginning the recession and the rate of draw down are adjusted, within limits, to mosquito control needs in the individual reservoirs.

The delay in the initiation of seasonal water level recession to about July 1 in the Tennessee River reservoirs has been demonstrated to be of

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the recession, with attendant increase in the acreage reaped, growth removal and larviciding. Recession during the late summer may be  
of the water in the lowered margins,  
ze to  
ison

reservoir affect all downstream reservoirs. Special design features and studies were required to incorporate water level management needs for malaria control into the scheduled operation of the nine main river reservoirs of the Tennessee River development. Stromquist (1935)

*Desirable features of water-level management*—A four phase water level management program for malaria control has been developed

normal operations required for meeting the basic purposes of the development, namely, flood control, navigation, and the generation of electricity. Refer to the graph in figure 3

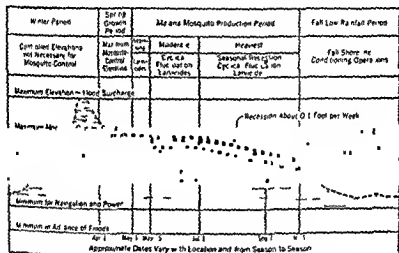


Figure 3

The marvel is that this has been accomplished without significant loss to any program interest. A very special effort has been, and is continuing to be, required of designing engineers and water level management planners and operators to satisfy the several program needs, but cooperative effort has been successful. Water level management is truly the foundation of the mosquito control program on TVA impoundages. The effectiveness and economy of the malaria-control program are largely dependent on the essential water level management features.

(1) *Flood surcharge*—The initial phase of the water level management of mosquito control on the main river projects is known as the flood surcharge and occurs in most reservoirs during the spring of the year as a result of flood-control operations. This consists of rais-

More recently Bishop and Gartrell (1944) described additional mechanical treatment in the form of deepening and filling and diking and dewatering of the shallow margins, which was applied in the Kentucky Reservoir of the Tennessee River development. These measures were used to eliminate permanently the largest mosquito breeding areas along margins where other measures under the conditions did not assure adequate mosquito control and the necessary capital investment for the major engineering works could be justified from the annual savings in application of routine repetitive measures. Larviciding on projects of the Tennessee River development, as well

*mosquito control for the moment*

*Emergency control measures*—At intervals emergencies occur on the Tennessee River reservoirs where departures either below or above the water level rule curves are necessary in meeting the primary purposes of the development. The departures are of a temporary nature, and where abnormal mosquito production develops as a consequence, the situation is met by larviciding and if the probability of malaria transmission appears imminent, DDT premise spraying is applied.

*Constant level pools and uncontrolled water levels*—Impoundages are encountered occasionally where the purpose of the project calls

the local situation and the malaria hazard involved. Alternative control measures may be used in the form of accelerated growth removal or control operations and application of larvicides, and DDT premise spraying if indicated. In special cases, permanent shore line improvement may have application or even land use restriction to daytime

mits all other normal uses of the area.

*Application of water level management to natural ponds*—The principles of water level management indicated herein would have ap

water level control structures. Any of the several phases of water level management might have some value even if the others could not be applied.

*Mutual interests—malaria control and wildlife*—The application of malaria control measures including water level management on im

Water level recession alone may be a very effective mosquito control measure, particularly if the reservoir has been thoroughly cleared and shore-line maintenance has been adequate. Assuming suitable water control structures at the dam, recession is a very definite possibility on most impoundages. This is not so true with periodic fluctuation since in flow must always be sufficient to refill the reservoir the approximate depth of the previous draw down.

The basic purpose of the storage reservoirs on the tributary streams of the Tennessee River requires schedules where water level needs for mosquito control are normally served by seasonal recession without periodic fluctuation. The size of the reservoirs with respect to summertime in flow and the purpose of the projects do not permit the normal scheduled use of periodic fluctuation.

*Periodic water level fluctuation without seasonal recession*—For various reasons, principally the limitation on marginal growth in invasion, periodic water level fluctuation without seasonal recession of the water level is most desirable in mosquito control. For reason

requires fluctuation without recession

*Seasonal recession with periodic water-level fluctuation*.—The four  
bas  
dis  
the  
level recession. A combination of these water level manipulations has

will extend below the lower limit of the growth invasion

*Supplementary control measures*—Water level management can be applied to both natural and artificial impoundages effective-  
ness being limited only by the degree to which combinations of me-  
chanical operations and water level manipulations can be made to  
reduce the larval habitat or directly control mosquito breeding

being applied along the margins after impoundage.



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pounded water need not be incompatible with the utilization of the lake for fish and wildlife development. Wiebe and Hess (1944) reported on the mutual interests which had been developed between wildlife conservation and malaria control on the Tennessee Valley Authority impoundages. A summary of these mutual interests is given

fe interests are mutually in  
The maintenance of a con

provides a maximum of area for production of fish food organisms. The control of woody plants, such as willow and buttonball, and certain aquatic plants, as lotus, cowhily, and lizardtail, reduces the mosquito

The four phases of water-level management set out above, plus a normal marginal growth removal operation, favor the production of valuable waterfowl food plants offering least objection in mosquito control.

drainage of depressions located along the margins minimizes the stranding of fish when the water is lowered.

From an economical point of view, malaria control programs on the impoundages usually provide a minimum application of larvicides which appears to be in line with conservation interests. The stocking or encouragement of mosquito larva predators, such as top minnows, as a malaria control practice, also favors wildlife interests through increasing fish food.

It has been demonstrated in the TVA reservoirs that diking and dewatering projects for malaria control may also be made to serve wildlife interests when operated to produce waterfowl food plants. The operation of these projects for malaria control

control

villages were kept absolutely free from any kind of vegetation on the margin, and despite most vigorous searches, not a single *fluviatilis* larva was collected from them. The incidence of malaria, however, was found to vary from place to place, and it later came to light that the reduction was inversely proportional to the extent of rice fields in and around the villages. Intensive investigations to determine other breeding grounds of *fluviatilis* than streams and channels eventually incriminated terraced rice fields as a very important source. The intensity of breeding was extremely low, and other species greatly outnumbered *fluviatilis*. But, as the extent of rice fields was very large, the total output was considerable. This phenomenon is not considered as having been brought about by any change in the breeding habits of *fluviatilis* after its most favored habitats, viz, streams and channels, have been rendered unsuitable by clean weeding, for such a phenomenon of change of habitat has never been described in the past and, if true, would strike at the root of the concept of species sanitation. Measures which included treatment of the rice fields were promptly followed by malaria reduction. In areas where no control was instituted rice fields continued to show *fluviatilis* breeding.

*Behaviour of fluviatilis—outdoor rest*—The failure of spray killing with pyrethrum twice a week led to studies on the behaviour of the adult *fluviatilis*, more especially on the extent to which the individuals rested in indoor places. It was obvious that outdoor resting was occurring, inasmuch as there was gross disparity in the numerical prevalence of the adult specimens collected every morning in the various stages of gonotrophic development. But even when the larval density was small, a sufficient number of adult *fluviatilis* was collected from indoor resting places every morning, several among them found on dissection to be infected. This led to a wrong assumption in the earlier stages that outdoor resting cannot but be of a small order. With the failure of spray killing with pyrethrum in reducing adult mosquito prevalence in sufficient numbers to bring

erated in a garden house at dusk. The only available indoor resting places were sprayed with an extra strong dose of pyrethrum extract a couple of hours after the release of the insects. During subsequent mornings and nights captures were made to see if there were any stained ones. As many as 10 percent were thus recaptured in all our experiments. These experiments conclusively proved that a good proportion of adult *A. fluviatilis* resorted to outdoor resting places and only some stayed indoors. More direct proof was also provided by the capture of 14 multiparous specimens of this species from the

provisional estimate  
to outdoor resting  
percent during the

## ACTIVITIES OF THE BOMBAY PROVINCIAL MALARIA ORGANIZATION, 1942-47

D K VISWANATHAN, M P H, *Assistant Director of Public Health  
(Malaria), Poona, Bombay Province*

*The problem*—Bombay, 1 of the 9 Provinces included in the Indian Union, has a population (1941) of 20,815,697 and an area of 76,389 square miles. The provincial birth rate is 33.2 per thousand, the death rate 23.0, and the infantile mortality rate 162 per 1,000 live births. Malaria death rate is about 1.5 per thousand, causing 33,000

Province. These, doubt, constitutes. While the present popular party, which was in power on an earlier occasion from 1937 to 1939, sowed the seed for the creation of a malaria organization, it was under the dynamic inspiration and advice of Maj Gen Sir Gordon Covell, then Director of the Malaria Institute of India, that the Bombay Provincial Malaria Organization was created on a permanent footing in 1942.

*First phase—Kanara district survey*—The first year was spent in cooperation with the Malaria Institute of India in an extensive survey of the whole district of Kanara, in the southernmost part of the Province, and in training of personnel. This survey revealed that malaria was hyperendemic, with spleen rates from 50 to 100 percent, except in a narrow coastal strip, and that the vector species is *A. fluviatilis* James. The natural infection rate was as high as 10 percent in most months of the year, and in some months every other specimen caught in nature was found infected. It is a preponderantly human feeder, with an anthropophilic index of 60 to 90 percent. This species was found during the survey to breed mostly in streams and channels with marginal vegetation.

is due to a complete lack of availability of larvicidal or insecticidal materials on account of the war. During this period nearly 100 miles of streams and channels in about 12

a year. The other day Komp put forth a plea for the correlation of infant malaria parasite rates and vector anopheles densities. In our study by such correlation month after month we determined that the density of 4 *fluviatilis* per 10 man hours is enough to maintain transmission in a community.

*Second phase—DDT.*—These activities marked the pre DDT period. The advent of DDT completely altered the picture. Preliminary trials with DDT in 1945 as an indoor residual spray showed such a total disappearance of *fluviatilis* for 2 months after the first round of spraying, that without any further loss of time a comprehensive scheme was submitted for malaria control in an area comprising 6,000 square miles in the two districts of Dharwar and Kanara, with about 1,200 malaria stricken villages and a population of a little over a million, involving the use of about 20 tons of DDT, at an estimated total cost of about 3 lakhs of rupees, or \$90,000. The pilot experiments in 30

sprayed villages. The new comprehensive scheme was duly put into effect in July 1946. The results showed that a dose of about 60 milligrams per square foot DDT indoor residual spray once in 2 months in the case of *fluviatilis*, and once in 6 weeks in the case of *culisfacies*, is efficacious in keeping them down below the critical density for transmission. For the first time in the history of malaria epidemiology, parasite rates and infant parasite rates exhibit signs of approximation to the zero point in hyperendemic areas in the tropics. The field is now open even in the tropics for plans for eradication of malaria, discarding the outworn advantages of pre-munition. I may here refer to the fears expressed by African workers that in economically backward communities having hyperendemic incidence of malaria, complete malaria control may, on account of loss of immunity, create an extreme hazard of severe epidemics at some future date when, because of economic depression, control measures may have to be abandoned. I would only say that the price paid for the acquisition of this immunity is so great even in such communities that it is an attitude of defeatism not to think of the same means of disease control in the tropics as in the temperate and the subtropical regions. The tropical people have as much right to modern advance in medicine as populations in any other part of the globe. A few illustrative data are furnished below to show the efficacy of the scheme.

(1) The anopheles densities in Kanara District have dropped to

(2) Spleen and parasite rates have dropped considerably. In several villages they are now less than 10 percent as against 50 to 100

colder months. On this basis the number of specimens that could survive at the end of a fortnight out of 100 adult individuals emerging every day of the week under different intervals of spacing of the spray killing programme was theoretically computed and a rational spacing of spray killing was evolved, viz., two consecutive days of spray killing separated by one and two sprayless days alternately rough in the year 1945 in killing had failed to give initial reduction in the in-

fluence of malaria was effected. For the conditions then prevailing, this method was considered the most economical for the control of rural malaria in Kanara district. With respect to towns where the concentration of population was larger, a judicious combination of antilarval control and twice a week spray killing with pyrethrum in areas most proximal to the breeding places was considered economically feasible. The cost in both cases at the then existing price levels was worked out as 1 rupee per head per annum (or about 30 cents).

*Time of entry and time of biting*—Studies made to determine the *atilis* showed of the night ng the early part of the night extremely hazardous and emphasizing the need for methods of personal prophylaxis. The foregoing experiments also showed that adult anopheles had a marked tendency for migration towards outdoor shelters at dusk and throughout the night irrespective of the state of gonotrophic development. It is only the freshly emerged individual or those which have just laid their eggs and are ready for further feeding that exhibit a movement towards houses.

*Races of fluviatilis*—It is of interest to record that while *A. fluviatilis* is a vector of such great intensity in Kanara district only a few miles outside the district it completely changes its habits, becomes mostly zoophilic, and a very feeble vector, if at all, for human plasmodia. There seems to be an inverse relationship between the density of *fluviatilis* and its anthropophilic index and malaria transmitting capacity.

*Infant malaria*.—In addition to these investigations a study was made of the prevalence of malaria amongst infants. The infant parasite rate averaged 10 percent. In a study of 432 infants practically once every month from their birth till 1 year thereafter, it was shown that malaria causes a great deal of the premature births and abortions and infantile mortality, but those infants who overcome the first and primary infection with plasmodia do not show any greater hazard of mortality during the rest of their infancy. The study also showed the fallacy arising out of assessing the season of transmission of malaria by an examination of infant parasite rates once or even twice

cation is the scientists' modern answer, with modern knowledge and to the extent to which communities can undertake it, it is certainly an excellent proposition, and Dr Aziz has shown us the way. For malaria reduction with practically all the mosquitoes.

It is only when

the mosquito transmits infection in bulk outdoors that the scheme will fail, even with a predominantly outdoor resting species such as *Anopheles fluviatilis*, but one which largely transmits infection indoors, the scheme has been shown to be extremely successful. Finally, I would refer to one important collateral effect, the possibility of prevention of human plague by the indoor residual program adopted for the control of malaria in the first instance. In the area covered by our scheme there has not been a single case of human plague during the last 18 months. It is no doubt too soon, but we have had in quite a few

case

not

rat burrows, since in the chain of plague transmission to man, man and flea must get contact outside the rat burrows, apparently the indoor residual spray is quite capable of taking care of it. A similar scheme has recently been sanctioned for another district with 1,000,000 population, at a cost of 3 lakhs of rupees, or \$90,000.

**Urban Malaria Control**—Among other calls for surveys, the most profitable one was made in 1944 in Greater Poona, an urban area which is the seat of government for several months in the year. Barber and Rice previously surveyed this area showed the extent of malarial endemicity, and indicated further lines of work. This noteworthy the most of the first

season. The total population benefitted by the scheme is estimated to be not less than 500,000, and the cost is thus only one tenth of a rupee per head per annum (3 cents) for a purely antilarval scheme. In this area copper cyanide is used extensively, entirely replacing paris green, which in 1944 was difficult to obtain. Biological control of the riverine breeding grounds by ponding, or converting them into deep impounded water by the construction of dams at intervals, is under active consideration.

**Studies on paludrine**—During the year 1946, an experiment was carried out which demonstrated the efficacy of paludrine in the chemotherapy of malaria in clinical prophylaxis and in treatment of the primary attack. Its greatest merit is its efficacy in the cure of a primary attack with a single administration of 300 milligrams, which revolutionizes treatment of malaria in rural areas. Two tablets a week at intervals of 3 and 4 days act as a good suppressive agent. The only disquieting feature is the appearance in the peripheral blood,

percent in the past. More than all, infant parasite rates are almost nil, about 0.8 percent in the sprayed villages as against 15 percent in unsprayed villages.

The formulation used consists of a 30 percent solution of DDT in medium kerosene extract. To this a 20 percent solution of soap is added, to make a mother emulsion with 25 percent DDT. This is diluted in the field to 5 percent. The dosage is roughly 1 quart for every thousand square feet, or 60 milligrams per square foot. In our experience, this formulation has given the most consistent results. The material is sprayed with an ordinary stirrup pump with a brass lance and a special nozzle, giving a conical shaped spray delivering about 600 cc. per minute, the size of nozzle orifice being  $3/64$  of an inch in diameter. The approximate cost of the scheme is about 6 to 8 annas (12-15 cents) per capita per annum for three rounds of spray in a year.

tion which contained some knock down ingredients such as thannite, and we did not have results comparable with the emulsion. We feel that the crystalline deposit of DDT which is obtained after the spray of emulsion, has perhaps the best insecticidal value. Addition of knock down ingredients may only serve to provide a sadistic satisfaction of seeing the carcasses of the enemy insects right under one's own eyes, but for disease control it makes no difference whether they die in the houses or out in the open.

I listened with rapt attention and admiration to the splendid ex-

Cyprus, and we are at present spending 100,000 rupees or \$30,000 on indoor residual spray. The program in Cyprus for eradication, if I heard Dr. Aziz right, would probably cost ultimately £200,000. After the subsidence of the first flush of enthusiasm for the eradication program, I feel that for Indian conditions, and perhaps for tropical conditions elsewhere too, the program of eradication is much too ambitious on account of its high cost. The hazards of the international situation may act as a bar to the complete prevention for all time of the importation of fresh anophelines. Again, in the tempo of modern scientific advance, who knows whether malaria could be controlled at a far cheaper cost in ways which we may not at present







## TROPICAL MEDICINE AND MALARIA

Dr. D. K. VISWANATHAN (India): I must confess that I have not got the personality to put a self-help plan in my province into operation at the moment, but there is another aspect to consider. For the local economic conditions of the people in Bombay Province in particular and in India generally, it makes very little difference if the money comes from the state or if the man provides for himself. I had a discussion about it with my minister the other day and his mind seems to be running in the direction of imposing a malaria tax so that sooner or later there will be a DDT program for every single village in the whole province.

after a course of treatment with paludrine, of a relatively large number of gametocytes. If, however, DDT control is established, there is no bar to the administration of this extremely useful, cheap and nontoxic drug.

*Future plans*—Five survey squads are now in service, one in each of five districts and in 3 years the survey of the entire province will be completed. On completion of the survey in each district, proposals are submitted for control schemes. We have recently submitted schemes

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control on present lines would cost about 5,000,000 rupees (\$1,000,000)

1,000,000

k of the Bombay

ular government

have all helped

in its rapid evolution and useful record of work. It is naturally anxious that in keeping with the present rate of progress in human activities the entire province should have within the next 5 years a network of control units and a only can progress be properly aspects of malaria may be conti

s briefly outlined

which is so vital

to the early achievement by the organisation of its future plans of development

#### ABSTRACT OF DISCUSSION

Dr LEWIS W HACKETT (Argentina) At the risk of keeping you from lunch for 2 minutes, I would like to ask Dr Viswanathan whether it isn't possible that the populations concerned could collaborate in malaria control. I suggested the other day at the World Health Organization Conference that the use of DDT should enter into family use in malaria populations. This has a distinct bearing also on Dr Soper's question of eradication. Should we eradicate or not in a D — — — — —

insects which we have including also some mammals like bats and mice which seem to disappear at the same time. The families are so enthusiastic about the results of this that while I understand in India you can't expect the families to provide much money, perhaps they could be induced to provide a little labor

## TROPICAL MEDICINE AND MALARIA

but also in the native fishponds, kept in their original state proved only by providing them with new sluice gates around the center field of the fishpond.

The growth of the bottom vegetation in the first years was After that period it did not grow at all, or grew in such small quantities that it was quite insufficient to feed the normal amount raised in a pond. It seemed as if the bottom of the ponds had become sterile.

The origin of the method of Walch and Reyntjes was in the part of Java, especially the region near the town of Pasoero Here the native fishpond owners raised fish by periodically drying ponds. There were no dangerous filiform algae, and there was always a sufficient amount of bottom vegetation for food.

What was the difference in Batavia, where, in contrast with Pasoeroen, the biological situation method had only very poor results a regards fish production? It seemed that a poor, porous sandy bottom will become exhausted and sterile after continuous drying over longer periods. When a bottom like this is irrigated by sterile sea water, there is no question of improvement. This was, to a certain extent, the case in Batavia.

In Pasoeroen the bottom of the ponds consisted of a rich compact clay, and the sea water was heavily silt laden. No wonder the periodic drying had no bad influence, on the contrary, the bottom was steadily improved.

In 1941 Dr. Markus (4) published a study on the character of bottom mud of salt water fishponds. His conclusion was that we want to exploit a fishpond according to the hygienic method. Walch and Reyntjes, the character of the bottom mud is of prime importance. There are ponds that, without intensive manuring, will never produce sufficient bottom vegetation to be of any importance as food for the fish. For those fishponds the dictum of an old fishpond owner is still valid that without surface vegetation there will never be good fish production.

In transmitting the experience of Pasoeroen to Batavia the mistake was made that the biological differences existing between the ponds in various regions were too little considered. It is not just possible to conclude that a biological (naturalistic) control method successful in one place will also be efficient in another place.

Batavia the method of Pasoeroen was first tried in a small number of ponds. The results in the first 2 years were so excellent that it was thought that the biological circumstances were the same as in Pasoeroen. The method was propagated for large areas in Batavia and along the north coast of Java, but after some time came to the conclusion that the conditions of the ponds were not the same in every place.

The fishery department started all kinds of experiments to increase the yield for the fish by manuring the water or the bottom.

# MALARIA CONTROL IN SALT WATER FISHPONDS IN JAVA

W J STOKER *Chief Section of Malaria Control Batavia, and Dr*  
J KUIPERS *Chief Malaria Engineer, Batavia*

During the Second International Malaria Congress in Algiers in 1930 the late Professor E W Walch (1) read a paper on a new biological sanitation method of salt water fishponds in the Netherlands Indies. Before this time several methods were tried to control the  
were too expensive to be economical.

The hygienic exploitation method of Walch and Reyntjes (2) had as its object to control malaria and also to preserve good fish production.

The principle  
water fishponds  
(*Enteromorpha*  
of the ponds and stimulation of the growth of the bottom vegetation  
(*Cyanophyceae*)

Walch and Reyntjes (1930) supposed that the surface vegetation, although it might be important as food for the fish, is not strictly necessary for good fish production in the ponds.

This surface vegetation consisting of long algae is dangerous, because it gives protection to the larvae of anopheles whereas the bottom algae give no protection at all to the larvae and provide the fish with a good food supply.

mental ponds near Batavia

We feel it our duty to report the various difficulties encountered.

As to the killing of the surface vegetation the periodical drying of the ponds was very successful. Soon all the dangerous weeds were gone and with them the breeding of *anopheles larvae*. The growth of the bottom vegetation was stimulated by leaving a small layer of water on the bottom of the pond.

During the first

Session 6 PRESENT PROPORTIONS OF THE GLOBE  
MALARIA PROBLEM

Monday, May 17—9 30 a.m. to 12 m  
Departmental Auditorium, Main Hall

POSTWAR MALARIA CONTROL IN GREECE AND ITS  
RESULTS ON BASIS OF EPIDEMIOLOGICAL DATA  
Prof G A LITADES and Dr G BELLOS, School of Hygiene,  
Athens, Greece

INTRODUCTION

Malaria, endemic in Greece since very ancient times, followed through the centuries and up to our era, not as an indifferent onlooker, all phases of the venturous career of the Greek nation. The first clinical and epidemiological observations of this disease, as well as the terms *tertia* (tertian), *quarta* (quartan), *σπληνομεγαλία* (splenomegaly), *θλάση καχεξία* (malarial cachexia), and other similar ones appear, as is known, in the works of ancient Greek writers and physicians. Modern epidemiological researches are placed

Greece at the top of malarious countries on the European continent. Except for a very few mountainous or insular areas and certain urban centers, the whole country is under the menace of malaria. Malaria mortality in Greece during the period 1931-35 was estimated at 73.7 deaths per 100,000 population, as against 5.5 in Italy—second among the most malarious European countries. In about the same period, malaria was the No. 3 death cause, regardless of age, and second in childhood after pneumonia. It was estimated during the said period that the number of persons attacked yearly by malaria averaged from 1 to 2 million (15 to 30 percent of the population) with a loss of 20 to 40 million man-days annually. During the same period, of the three parasite species encountered in the country, *Pl. falciparum* prevails during the late summer months and fall and in greater portion during years of increased epidemicity. *Pl. vivax* occurs in spring and early summer and *Pl. malariae* in all seasons but often in winter and in a greater proportion with *Pl. vivax* during of low epidemicity. Mixed infections are very frequent the eight anopheline species occurring in the country, those suitable for transmission of the disease, in order of importance *An. clutis*, *A. superpictus*, and *A. maculipennis* (var. *typicus*) and

gave more satisfactory results but the expenses were as high as the profits

In 1939 the fishery department decided to stop the periodical drying of the ponds. As soon as the dangerous weeds appeared on the water surface they were collected by mancraft and put in small heaps in the ponds, covered with a layer of earth to prevent their floating on the surface.

This method is only a compromise between malaria control and fish breeding because the yield of fish is still small in comparison with the production of the formerly malaria dangerous fishponds. The fishery experts are now studying the more intimate biological processes which occur on the bottom surface and in the other layers deeper in the earth. It is possible that in the future each complex of fishponds will have its own sanitation method.

With our present knowledge we are anxious to allow the construction of new ponds especially in densely populated areas. It must be regretted that during the Japanese occupation of the Netherlands the construction of new fishponds, water ponds can never be endemic malaria on

### SUMMARY

The hygienic exploitation method of salt water fishponds in Indonesia as described by Walch in 1930 has not fully answered expectations. As a malaria-control method it was successful, but from the point of view of pisciculture it was a big disappointment. The probable reasons are recorded and also the attempts made to effect a good coordination of malaria control and sufficient fish production.

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mortality and morbidity data for the areas included in the program with those of other areas proved very satisfactory.

It should, however, be noted that to obtain these results, hard and strenuous efforts had to be made, and the major portion of the population placed under protection from malaria by the program outlined belonged to urban areas (80 percent). Any attempt to extend these measures to rural areas where the existing malaria problem was more acute, came up against the high cost that their application would involve. It can, therefore be stated that the malaria problem in small rural areas of Greece had remained by that period unsolved.

There followed the years of war and enemy occupation (1941-44) during which due to existing unfavorable conditions the Malaria Control Service was able to develop very limited activity.

During this period malaria incidence, aided by various favorable factors (undernourishment, hardships, mass movement of population forced inactivity of the service etc.) returned to its previous levels, and in 1942 it reached one of the highest peaks in its recent history (10).

### POSTWAR ANTIMALARIA ACTIVITY

On the liberation of the country (October 1944) drastic measures were taken to reorganize the Malaria Service then existing in a latent condition as a result of the enemy occupation. Under this reorganization, the Service retained its original structure.

Substantial changes were made about the middle of 1946 by the inclusion in the malaria control organization of Prefectural Health Centers (17). This action was made necessary due to the extended scope that the malaria program presently assumed.

During the 1945 malaria season the control activity returned to prewar levels. The 150 local malaria programs, carried out in that year all over the country, that gave protection to approximately 1 000,000 of population, were chiefly based on larva control by classical methods.

Meanwhile, the importation of small quantities of DDT in October 1944 and the subsequent verification in our laboratories of its insecticidal value that had already been demonstrated by previous researches (11, 12) produced on us the vivid impression that by the new discovery the malaria control work in this country entered a new hopeful phase, especially in regard to the malaria problem of rural areas (13, 14). This direct conclusion was evidently reached through the knowledge of ecology of Greek anopheline species.

As soon as the first quantities of the new insecticide were made available, we started in the early summer of 1945 a series of experimental applications as an adult control measure in three groups of

*subalpinus*) All these three are rather domestic species. *A. elutus* breeds chiefly in seashore swamps, *A. maculipennis* in small or large swamps and in miscellaneous water collections, and *A. superpictus* in the numerous torrents overrunning the country.

The transmission period for this disease lasts from May to October. The occurrence of mass relapses in the spring (April to May) is frequent.

Malaria studies in Greece in modern times were inaugurated by the works of Savvas and Kardamatis (1905) (1). A special advancement in these studies was attained by the establishment of the Athens School of Hygiene in 1930, with a special Department of Malariology and Tropical Diseases (2). The cooperation with the School of a mission of the Rockefeller Foundation (M. C. Balfour (3, 4, 5), D. E. Wright (1931-38)), assigned to Greece at that period and assisted later by other prominent Foundation workers (Barber (6, 7), Shannon (8),

malaria control program and organize a special service for its operation.

Under this program the country was divided into 10 malaria regions, to each of which a doctor malarialogist was assigned with an adequate number of well trained malaria inspectors. All other personnel was naturally recruited locally, according to requirements. The central laboratories and general direction of malaria control activities were stationed in the Department of Malariology and Tropical Diseases of the Athens School of Hygiene, which by its director and traveling technical personnel, malarialogists, entomologist, and engineers, issued the necessary instructions in the field and made regular inspections of the work under way.

Between the years 1937 and 1941 this special service thus established, carried out on a progressive scale a number of local malaria control programs in various areas of the country in cooperation with the regional public-health services and local authorities. The population placed under protection from malaria by these programs amounted to 1,150,000 in 1940 (16).

The measures used were mainly based on a systematic antilarval work (17).

was a systematic use of malaria drugs made as a preventive measure, except by the army and certain organizations.

The results obtained in this work, as indicated by a comparison of endemoepidemicity indices (spleen and parasite indices of children of school age and parasite index of newborn babies), and of

a view to covering at least its greater part by the end of May or early part of June.

terminated by Headquarters in cooperation with the regional services, on the basis of the prevailing anopheline species in each particular

interior walls, and no accessible room in the living quarters or out buildings is precluded from spraying. In this way, the simultaneous control of other domestic pests is obtained, with no considerable charge and the house spray method is thereby rendered still more acceptable and popular.

The uniform dose of active ingredient used is 1.8 gram per square meter. This quantity, as concluded from our observations (15), generally insures protection for over 6 months.

The spray equipment in use chiefly consists of hand sprayers. Spraying is done by small mobile teams, composed of 1 foreman and 2 to 3 spraymen recruited locally and properly trained. The Provincial Malaria Inspector has 3 to 7 such teams under him, and his work is supervised by the Chief Malaria Inspector of the Prefecture, who in turn reports directly to the Chief of Prefectural Health Center and is under the technical control of the Regional Malariologist of the area.

A technical and financial account of the program of house spray with DDT for 1946 and 1947 is shown in table 1.

The total cost of residual spray program in 1947 amounted to 6,541.3 million drachmas (Drs 6,000= \$1) or approximately \$0.31 per protected capita.

TABLE 1—*Technical and financial record of residual spray program for 1946 and 1947 in Greece*

| Item No | Description (Items and representative rates) | 1946       | 1947        |
|---------|--|------------|-------------|
| 1       | V. per gram of                               | 4,139      | 5,780       |
| "       | "  | 497,300    | 663,000     |
| "       | "  | 2,024,400  | 2,988,000   |
| "       | "  | 93,000,000 | 130,000,000 |
| "       | "  | 2,852,000  | 3,490,000   |
| "       | "  | 3,602,700  | 6,232,900   |
| "       | "  | 4.25       | 4.35        |
| "       | "  | 894        | 983         |
| "       | "  | \$60       | 1,000       |
| "       | "  | 51         | 78          |
| "       | "  | 74.6       | 18.8        |
| "       | "  | 6.7        | 20.2        |
| "       | "  | 18.5       | 60.8        |
| "       | "  | 67,885     | 102,354     |
| "       | "  | 42         | 40          |
| "       | "  | (7)        | \$0.31      |

existing conditions in this country and we therefore considered it advisable, in the course of this experiment, to engage not only in the entomological and epidemiological approach to the problem but also in the technical and economic outlook of the method (15). It is noteworthy that the house spray technique established during this experiment has not required any substantial changes ever since.

Under these circumstances, the use of DDT in this country on a large scale depended exclusively on the availability of the necessary material and equipment and the appropriation of necessary funds.

The enthusiasm and keen interest of the director of the UNRRA Sanitation Section in Greece, Col D E Wright, played in this matter an important and decisive part.

Indeed, this very good friend of Greece and malaria campaign

to obtain the necessary appropriations from the Government. Throughout the development of the malaria program he placed all his valuable experience at the disposal of the Service and in addition

the principal pioneers of the malaria-control work accomplished in the country during that period.

#### BRIEF OUTLINE OF MALARIA PROGRAM OPERATED IN 1946 AND 1947

spray with DDT was applied only during the latter part of 1945 and on a limited scale.

to the extended malaria program carried out during that period. We will therefore confine ourselves only to the most important relative data.

The methods applied are as follows:

(1) *House spray with DDT*—This was the selected method for the protection of rural areas with a population not exceeding 2,500. These areas, according to the 1940 census, comprise over one half (53 per cent) of the population of Greece and are most heavily stricken with malaria.

The operation of the residual spray program starts in March with

## TROPICAL MEDICINE AND MALARIA

TABLE 2—Technical and financial record of ground larviciding in Greece for 1946 and 1947

| Financial record of ground larviciding in Greece for 1946 and 1947   |  |                                     |                       |                |                                     |                       |   |  |  | MEDICINE AND MALARIA |  |  |  |  |  |  |  |  |  |
|--|--|-------------------------------------|-----------------------|----------------|-------------------------------------|-----------------------|---|--|--|----------------------|--|--|--|--|--|--|--|--|--|
| Item No  | Description  | 1946:                               |                       |                |                                     | 1947                  |   |  |  | Total for 1947       |  |  |  |  |  |  |  |  |  |
|  |  | Larva control operated individually |                       | Total for 1946 | Larva control operated individually |                       | Combined larvicide and house spray <sup>1</sup> |  |  |                      |  |  |  |  |  |  |  |  |  |
|  |  | Adequate protection                 | Inadequate protection |                | Adequate protection                 | Inadequate protection |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 1  | Local larva control programs                               | 147                                 | 26                    | 74             | 113                                 | 23                    | 647   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 2  | Areas included in the local programs                       | 258                                 |                       |                | 121                                 |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 3  | Population of areas included in the local programs         | 1,342,000                           | 171,000               | 723,000        | 1,393,000                           | 90,000                | 633,000   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 4  | Average population of areas included in the local programs |                                     | 6.570                 |                |                                     | 3,920                 |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 5  | Average operational duration of local programs             |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 6  | Final solution emulsion or suspension used                 |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 7  | Analysis of material used                                  |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 8  | DDT technical grade  | 6,140                               |                       |                | 12,150                              |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 9  | Pure oil   | 21.6                                |                       |                | 12.9                                |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 10   | Estimated surface of swamps sprayed                        |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 11   | DDT solution or emulsion                                   |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 12   | Pure green   |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 13   | Average surface sprayed per man day                        |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 14   | Cost per square kilometer sprayed                          |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 15   | Cost per protected person (column 3) <sup>1</sup>          |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 1. Certain incomplete technical data for that year were roughly estimated. Moreover the data for one region (Crete) were completely omitted. |  |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| 2. The population of these columns was included in the corresponding data of table 1.  |  |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
| No significant data are available for an estimate of 1946 cost.  |  |                                     |                       |                |                                     |                       |   |  |  |                      |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 5,687,300            |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 4.9                  |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 248                  |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 2.2                  |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 299,710,000          |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 90                   |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 2.5                  |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 84,334               |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 2,620                |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 535                  |  |  |  |  |  |  |  |  |  |
|  |  |                                     |                       |                |                                     |                       |   |  |  | 11.7                 |  |  |  |  |  |  |  |  |  |

<sup>1</sup> Certain incomplete technical data for that year were roughly estimated. Moreover the data for one region (Crete) were completely omitted. The population of these columns was included in the corresponding data of table 1. No significant data are available for an estimate of 1946 cost.

The actual participation of the general public in the operation of this method was very satisfactory, which is borne out by the fact that practically no room remained unsprayed (less than 15 percent for 1946 and 1947)

*Larva control by ground methods*—This was carried out for the protection of urban and urban rural areas, where house spray with

writers (G B)

over the older

it was applied

throughout the country in 1947 (5 parts of 26 percent DDT emulsion to 1 part of 20 percent DDT in Velsicol NR 70, addition of water to obtain a 1:1000 mix, and larviciding done about every 12 days with an average dose of 0.02 grams technical grade per square meter of breeding area)

A technical and financial account of larva-control program for 1946 and 1947 is shown in table 2

*Larva control from airplanes*—While the effectiveness in this coun-

addition the miscellaneous handicaps<sup>1</sup> faced by the Service, operating under actual warfare conditions did not permit an adequate rationalization of the method.

are the corresponding data of a similar survey carried out in the fall of 1947. In these papers, a very detailed analysis is made of the foregoing data, in correlation to the methods used in each particular case, along with a comparison with the corresponding data of prewar years.

In table 4 are included collectively the parasite indices of children of school age for 1946 and 1947 by like groups for easier comparison. These indices cover malaria stricken areas, urban and rural, scattered all over the country and with varied malaria conditions, that were protected during these years by one or more methods. Where possible, corresponding average indices collected over the period 1933-45 are given for comparison.

Although we by no means ignore the disadvantages of this statistical illustration, the extent of the index decline observed and the steadiness of the phenomenon leave no doubt regarding its value and interpretation. In fact, as shown in table 4, the parasite indices of 1946 and 1947 are, on the average, about one seventh of the 1945 indices, and the 1947 indices are about one seventh of the 1946 indices or one

seventy ninth of the corresponding average indices of previous years. The baby parasite index in 57 protected areas (558 blood smears) in 1946 was found to be 0.18 percent and in 1947 in 58 also protected areas (645 smears) reduced to zero.<sup>1</sup>

The spleen index of school age children declined considerably during these years. In 19 areas of the Attica Prefecture, where this index was taken personally by us, there was a drop in the average spleen index from 25 percent in 1945 (observation year) to 18 percent in 1946 and 12.3 percent in 1947.

It is worthy of notice that of the 32 positives found in 1947, of a total 15,059 (school age) smears examined by that time, not a single one showed high density of parasites. In half of them (16 of 32) the number of parasites rated 1 or 2 per 200 optical fields (thick smear). Most of the parasites bore apparent intense morphologic signs of degeneration. The species proportion was found as follows: *Pl. vivax*, 24 (75 percent), *Pl. malariae*, 4 (12.5 percent), and *Pl. falciparum*, 4 (12.5 percent). All this provides convincing evidence

that the present situation was of no importance. The epidemiological data contained herein, one can venture a *grosso modo* estimate of malaria in the country. The parasite index for 1946 was 0.18 percent, and for 1947 it was 0.01 percent. The number of cases was 18 in 1946 and 9 in 1947. The number of deaths was 1 in 1946 and 0 in 1947. The number of cases was 18 in 1946 (18), and in the following year there was a still further reduction.

<sup>1</sup> Examined to April 7. After examining also the other blood smears of babies collected during the 1947 epidemiological survey the final results are briefly as follows: areas surveyed, 70; blood smears collected, 823; positives, 0; parasite index, 0.

Table 3 gives a technical and financial account of the 1946 and 1947 air spray program

TABLE 3—*Technical and financial record of air spray program in Greece for 1946 and 1947*

| Item No | Description                                      | 1946 <sup>1</sup> | 1947        |
|---------|--|-------------------|-------------|
| 1       | expenses treated during the five previous months | 100               | 189         |
| 2       | " " " " " "                                      |                   | 370         |
| 3       | " " " " " "                                      |                   | 1 96        |
| 4       | " " " " " "                                      |                   | 11          |
| 5       | " " " " " "                                      |                   | 1           |
| 6       | " " " " " "                                      |                   | 6.2         |
| 7       | " " " " " "                                      |                   | 28.5        |
| 8       | " " " " " "                                      |                   | 3 250       |
| 9       | " " " " " "                                      |                   | 3 15        |
| 10      | " " " " " "                                      |                   | 47 220      |
| 11      | " " " " " "                                      |                   | 2 285       |
| 12      | " " " " " "                                      |                   | 0 872       |
| 13      | " " " " " "                                      |                   | 310         |
| 14      | Cost per square kilometer sprayed                | in U.S. dollars   | 9 430<br>65 |

<sup>1</sup>Some of the 1946 data are incomplete

cent of this sum was drawn from the malaria control budget, 41.2 percent covers the estimated cost of UNRRA supplies, amortization on the malaria airplanes, vehicles, and miscellaneous other equipment brought by UNRRA, and finally, 17 percent is estimated to represent the Ministry of Air share of the cost (personnel salaries, repairs to aircraft, etc.)

solvents, and freight, 42.1 percent, transportation expenses and operational cost and moving of vehicles, 12.5 percent, special air spray expenditures, 6.6 percent, miscellaneous expenses, 2.2 percent. The total cost among the population for residual spray and ground for 1946 was \$0.40

#### EFFECT OF THE APPLIED PROGRAM ON MALARIA INDICES

In a monograph (18) by the writers and their collaborators, all epidemiological data (spleen and parasite index of school age children, parasite index of babyhood) are given of an extensive country wide survey made in the fall of 1946, and, in another paper (in print)



## CONCLUSIONS

From the foregoing and other data published by the writers it is concluded that malaria in Greece had a surprising decline in the years 1946 and 1947 for the first time in its history

For a more accurate interpretation of this phenomenon, it is necessary that the following factors be taken into consideration

1 A periodic rise or drop in malaria indices is noted in Greece but never has there been such or even a similar big decline, so far as is known

2 Outbreaks of malaria epidemic are observed every 3 to 5 years

3 The last epidemic outbreak in the country was in 1942

4 " " prevailed during the 3 successive years 1946 and 1947, which from all possible indications must be considered as years of approximately average epidemicity

5 The political unrest in the country during 1946 and 1947 and the ensuing economic conditions did by no means prove to be unfavorable factors in the spread of malaria

On the basis of the foregoing it can be concluded that the astonishing phenomenon but is mostly due during this period

This conclusion is further corroborated by the generally observed absence of malaria carrier mosquitoes from populated areas where malaria control measures were carried out, as well as by the fact, definitely established through a few but very careful surveys, that this phenomenon did not occur in those areas where such measures were either not taken or considerably delayed

The benefit derived as a whole seems to be nearing the theoretical

operated, can result in a break in the malaria chain in said areas within 1 to 2 years, as demonstrated by surveys in Attica, where this method was only practically used. From surveys in Attica, it was also found that house spray with DDT can also insure protection from malaria even for residents of the sprayed settlements that make an overnight stay outdoors

surrounding areas as are in proximity with the breeding places

TABLE 4.—Comparative parasite indices of school age in Greece in areas protected during 1946 and 1947 with other relative data where available

| Groups of areas  | Areas and total population of group                                 | Parasite index for previous years (1 to 10) |                        | Parasite index for 1946        |                 | Parasite index for 1947        |                 | Parasite index ratio for the different periods |
|------------------|---|---|------------------------|--------------------------------|-----------------|--------------------------------|-----------------|--|
|                  |   | Persons examined                            | Average parasite index | Persons examined               | Parasite index  | Persons examined               | Parasite index  |  |
|                  |   |   |                        |                                |                 |                                |                 |  |
| Group 1          | Comparison of indices of previous years with those of 1946 and 1947 | Number<br>99 (191,000)                      | Percent<br>16.4        | Number<br>29,979               | Percent<br>2.64 | Number<br>7,800                | Percent<br>0.19 | 86 11 1  |
| Group 2          | Comparison of indices of previous years with those of 1946          | 27 (777,000)                                | 15.9                   | 5,647                          | 2.77            | --                             | --              | 20 1   |
| Group 3          | Comparison of indices of previous years with those of 1947          | 35 (129,000)                                | 17.6                   | 2,327                          | 2.34            | 4,047                          | 27              | 65 1   |
| Group 4          | Comparison of indices of 1946 and 47 with those of 1947             | 37 (37,100)                                 |                        | 2,012                          | 2.34            | 2,304                          | 17              | 87 1   |
| Group 5          | Comparison of 1946 and 47 with those of 1947                        | 30 (21,500)                                 |                        | 1,604                          | 1.64            | 908                            | 22              | --   |
| Group 6          | 1946 indices with no comparable data.                               | 19 (14,500)                                 | 16.6                   | 12,530                         | 1.79            | 115,059                        | 21              | 70 8.5 1                                       |
| Total or average |   | (131 areas population 397,709)              |                        | (163 areas population 327,800) |                 | (177 areas population 371,800) |                 |  |

Population in parentheses.  
 1 Examined in Apr. 7, 1945. After examining also the other blood surveys of school age, collected during the 1947 epidemiological survey the final results are briefly as follows.  
 areas surveyed 236 blood surveys collected 19,931 positives 41 general parasite index 0.21 percent.

## L'ETAT ACTUEL DU PROBLEME PALUDEEN AU CONGO BELGE, RESP. EN AFRIQUE CENTRALE<sup>1</sup>

J. SCHWARTZ, *Ancien Médecin Inspecteur, Directeur de Laboratoire et Chef de Mission au Congo Belge, Membre de l'ancienne Commission du Paludisme de la Société des Nations, Professeur d'Hygiène tropicale à l'Université de Bruxelles*

Nous nous occupons du problème culicido paludéen au Congo Belge depuis 1928, soit depuis 20 ans en qualité de médecin du Gouverne-

ment fut présentée au 2<sup>me</sup> Congrès International du Paludisme à Alger 1930 (1). Au 3<sup>me</sup> Congrès, celui d'Amsterdam 1938, nous avons présenté un résumé succinct sur le Paludisme endémique des noirs, résumé basé sur l'examen de 8557 indigènes de tous les âges et de diverses régions (2). Depuis lors nous avons pu compléter nos connaissances d'alors sur notre problème par de nouvelles recherches faites en 1939 et en 1945-1946, aussi bien sur le Paludisme endémique afebrile que sur le Paludisme épidémique fébrile des noirs, ainsi que sur la limite altimétrique du paludisme et sur certains anophèles spéciaux, notamment sur ceux des hautes altitudes. Ces nouvelles recherches ayant également été exposées dans une série d'études, dont nous ne citerons ici que trois (3, 4 et 5), nous nous bornerons, dans la présente étude, également à leur bref résumé. Mais pour mieux faire ressortir l'intérêt de nos nouvelles constatations, nous devons préalablement rappeler les anciennes, en esquisant ici un tableau schématisé du Paludisme centro africain.

### PALUDISME ENDEMIQUE AFEBRILE ET PALUDISME AIGU FEBRILE

Il existe en Afrique centrale deux modalités de paludisme : paludisme endémique, afebrile, des autochtones, des noirs, et paludisme aigu, fébrile, des immigrés, des Européens. La place nous manquant ici pour entrer dans les détails du mécanisme de l'immunité, ou de la prémunition, dans le paludisme, nous nous bornerons à la constatation du fait que les autochtones de l'Afrique centrale possèdent une tolérance spéciale envers leur infection paludéenne. Tandis que les immigrés, les Européens, réagissent à leur infection par la fièvre et par les diverses complications connues, dont l'hémoglobinurie.

In so far as the effectiveness of air spray in Greece is concerned, the data available are, as stated, inadequate to permit a definite opinion on the matter. However, we are gradually more and more convinced that to make this method really effective in Greece, a systematic look-

will  
a few

If this favorable prospect comes true, the whole malaria control work in Greece could be assigned to small, quick-moving teams that would take care of any area where malaria cases were reported

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*shalli* var *moucheti* (= *A. moucheti*) La répartition de ces anophèles est différente suivant les régions tantôt on en trouve plusieurs ensemble et tantôt uniquement l'une ou l'autre. Mais toutes deviennent très rares vers l'altitude de 1600 à 1700 mètres et disparaissent complètement vers 1800 mètres. On trouve encore des anophèles à 1800 mètres et même à 2000 et à 2200 mètres, mais il s'agit d'anophèles spéciaux des hautes altitudes, do non transmetteurs du paludisme—jusqu'à la preuve du contraire—et notamment de *A. christyi*, *A. lingi*, *A. transiensis* (= *A. demeilloni*) et *A. garnhami*. La limite entre les anophèles transmetteurs du paludisme et les non transmetteurs est donc en même temps la limite altimétrique du paludisme ce qui se comprend.

### LE PALUDISME DES EUROPÉENS

Il est inutile de dire que c'est le paludisme endémique des noirs est la source du paludisme aigu des Européens. Il est par conséquent difficile et même impossible de lutter contre le paludisme des Européens sans lutter préalablement ou simultanément contre le paludisme noirs.

### LE PALUDISME ENDEMIQUE ET ÉPIDÉMIQUE DES NOIRS

Dans la règle le paludisme endémique des noirs est afebrile. Dans certaines régions, hyperendémiques, ce fait est vraiment frappant. Maintes fois nous avons trouvé jusque 100% de parasites chez cinquante ou une centaine de nourrissons, âgés de 3 mois à parasites de plus, par un très grand nombre de parasites de même de toutes les trois espèces, dont des dizaines de gamétocytes croissants y compris, dans une goutte épaisse et même dans un Et tous ces enfants se portaient parfaitement bien ni fièvre symptôme morbide.

Mais on sait que, quelquefois, pour une cause ou pour une les noirs perdent leur résistance aux parasites paludéens tractent un accès fébrile. Ces cas s'observent même chez les mais surtout chez les très jeunes enfants, ce qui est facile à constater. Le diagnostic exact de ces cas fébriles n'est pas toujours facile.

Certaines personnes attribuent la grande mortalité infantile au paludisme, mais il n'existe sous ce rapport aucune statistique. Les enfants noirs sont atteints d'un fort parasitisme intestinal. Les enfants noirs ont des troubles gastro-intestinaux variés. Or, les paludéens se trouvent chez presque tous les enfants noirs. C'est ainsi que, chez les malades de n'importe quelle origine, surtout à des maladies gastro-intestinales ou à des infections respiratoires.

Dans une étude actuellement sous presse nous avons constaté que l'acutisme de la malaria est la cause de la malaria aiguë et de la malaria chronique.

## LES TROIS ESPECES PALUDEENNES EN AFRIQUE CENTRALE

Il est inutile de dire que c'est la Tierce tropicale, *P. falciparum*, qui est l'espèce dominante dans le centre de l'Afrique. Mais les deux autres espèces y existent également. Seulement la répartition des espèces paludeennes est différente dans le paludisme endémique des noirs et le paludisme fébrile des Européens

*parum*, puis également par *P. malariae* et enfin aussi par *P. vivax*. A partir de l'âge de 2 à 5 ans suivant les régions on commence à observer un phénomène inverse : la disparition de *P. vivax* d'abord et de *P. malariae* ensuite, de sorte que chez les adultes il ne reste dans la

est le plus rare et le plus fugace et *P. falciparum* le plus commun et le plus stable. *P. malariae* occupe une place intermédiaire entre les deux.

Dans le paludisme aigu des Européens il s'agit presque uniquement de *P. falciparum*. Les infections à *P. vivax* sont très rares\*. Quant à *P. malariae* nous n'en avons jamais vu chez les Européens en Afrique. La "quarte" semble donc être une "survivance" du paludisme endémique et son rôle reste bien mystérieux.

## L'ABSENCE DE PALUDISME ENDEMIQUE DANS CERTAINES REGIONS ET LA CAUSE DE CETTE ABSENCE

Le paludisme existe pratiquement partout dans l'Afrique inter tropicale mais non absolument partout. En effet il existe certaines régions ou, du moins certaines localités, où l'on ne trouve pas de paludisme du tout. Ce sont les agglomérations indigènes situées sur de hauts plateaux ou hautes collines au dessus de 1800 mètres d'alti

et leur répartition

## LES ANOPHELES DU CONGO BELGE

Il y existe de nombreuses espèces et variétés—surtout suivant les régions—mais ce ne sont que quatre espèces qui sont connues comme transmetteuses du paludisme. Ces espèces sont, dans l'ordre de leur fréquence et importance *A. gambiae*, *A. funestus*, *A. nabi* et *A. mar-*

\* Mais chez les colons rentrés en Europe les rechutes sont dues dans la règle à *P. vivax*.

vant dans des régions plus basses, dans des régions à paludisme endémique. Ces travailleurs, non prémunis contre les méfaits des parasites paludéens, ne tardaient pas de contracter le paludisme aigu fébrile avec toutes ses complications, à l'instar des Européens. Nous avons pu étudier le résultat d'une de ces 'émigrations' en 1939 (6).

Mais c'est surtout dans le Ruanda Urundi que le problème du paludisme aigu, épidémique et fœbrile, chez les noirs, est devenu de plus en plus important et urgent à résoudre. La cause de cette éclosion assez subite d'épidémies de paludisme dans diverses régions du Ruanda Urundi est un peu spéciale.

Il s'agit d'un pays montagneux et accidenté, où les hauts plateaux et collines sont entourés de profondes vallées marécageuses. Les indigènes y habitent sur les collines dont ils cultivaient le sommet et les pentes. Mais les sécheresses périodiques provoquaient des disettes fréquentes de vivres et parfois même de vraies famines. Pour remédier à cette situation le Gouvernement a eu recours à la bonification des vallées marécageuses en les drainant et désherbant et en les faisant ensuite cultiver. Cette excellente mesure humanitaire au point de vue agricole a eu des conséquences désastreuses au point de vue paludéen. D'autant plus que le drainage a substitué aux divers *Culicidés* de ces marais (surtout les *Taeniorhynchus* (*Coquillettidia* *Mansonioides*), des anophèles et surtout *A. gambiae* et *A. funestus*. De sorte que la cultivation de ces vallées ne tarda pas de provoquer de vraies épidémies de paludisme aigu avec un certain pourcentage de cas mortels, dus soit à des accès pernicieux, soit même à l'hémoglobinurie.

Nous avons pu étudier plusieurs de ces épidémies lors de notre mission au Congo en 1946 dont les constatations furent exposées dans un mémoire actuellement sous presse (5)

La place nous manqua ici pour exposer les divers *d'ails* observés dans ces diverses épidémies, détails curieux, intéressants et importants. Nous nous bornerons à en signaler bien brièvement les deux constatations suivantes : une, parasitologique et une autre, épidémiologique.

## CONSTATATIONS PARASITOLOGIQUES

En examinant la population d'une agglomération "ordinaire" c'est-à-dire à paludisme endémique, on constate

- (1) Une beaucoup plus faible proportion de parasités chez les adultes que chez les enfants,

lisme épidémique, dans  
peu plus tard dans le  
stade sub aigu et déjà anémique, nous trouvons le même pourcentage  
de parasites—et parfois même plus grand—chez les adultes que chez les  
petits enfants. De plus, autant de parasites de *P. malariae* et de *P.*  
*vivax* chez les adultes que chez les enfants.

## CONSTATATIONS ÉPIDÉMIOLOGIQUES

Une de ces agglomérations de hautes altitudes à paludisme récent fut examinée par nous en 1939 et réexaminée en 1946. Nous avons trouvé la première fois une proportion de parasités plus faible que la deuxième fois, mais par contre une proportion plus forte de cas fébriles parmi les parasités. Ce qui veut dire que le paludisme aigu passe spontanément par des stades sub-aigu et chronique pour devenir "endémique". Mais ce processus d'immunisation spontanée a été accompagnée entre temps d'un certain nombre de morts par des accès pernicieux et par l'hémoglobinurie, sans parler d'un grand nombre de malades.

## LE PROBLÈME ACTUEL DU PALUDISME AU CONGO BELGE ET DANS LE RUANDA-URUNDI

Comme tout le monde, nous considérons jusqu'à ces dernières années le paludisme comme la maladie tropicale la plus grave pour les Européens, comme le plus grand obstacle à leur installation en Afrique centrale. Mais nous faisons peu de cas du paludisme, quand il s'agit des noirs. Et si l'on pensait à quinquiner les noirs, c'était pour faire disparaître leurs parasites nocifs pour les blancs. Mais depuis quelques années le paludisme est devenu—pour des raisons esquissées plus haut—une maladie grave et même mortelle également

phylactiques antipaludéennes connues, toutes excellentes en théorie mais dont l'application est bien souvent irréalisable. Dans de très grands centres européens on envisage—et pratique—la prophylaxie antilarvaire en grand, prophylaxie très coûteuse et de longue haleine. Mais partout ailleurs cette prophylaxie est irréalisable.

Dans les contrées européennes à paludisme endémique l'infection n'a lieu que durant les périodes estivales. L'immunisation y est de trop courte durée pour empêcher des poussées épidémiques annuelles. Il ne s'agit en somme ni de vrai paludisme endémique ni de vrai paludisme épidémique, mais de courtes poussées annuelles de paludisme aigu après de plus longues accalmies annuelles. Tandis qu'en Afrique centrale l'infection est permanente, ce qui a comme résultat une im-  
un vrai paludisme endémique,

durant les poussées aiguës n'est ainsi pas applicable en Afrique centrale, inapplicable et même, peut être, indésirable pour des raisons exposées plus haut. Certes, quand il s'agit d'un cas de paludisme aigu ou d'une épidémie de





## PRESENT PROPORTIONS OF THE MALARIA PROBLEM IN THE NEARCTIC REGION

JUSTIN M. ANDREWS, *Scientist Director, Deputy Officer in Charge Communicable Disease Center, United States Public Health Service, Atlanta, Ga.*

With the exception of central and southern Mexico and Central America, the Nearctic Region includes all of North America Green land and the interjacent islands (1) Most of the malaria in this region during recent years has been in the United States The following discussion is based primarily on the present dimensions of the malaria problem in this country, together with supplementary information concerning recent malaria morbidity and mortality reported from the bordering Provinces of the Dominion of Canada and States of the Republic of Mexico

*Current trends in Nearctic malaria prevalence*—Paludism in the United States is a continuing disease This is borne out by the historical records extending back to the early days of settlement (2) Carter (3) Childs

tions and the importation of Negroes from Africa introduced large numbers of parasitized individuals to North America Wet rice culture the clearing of wooded lands for crop purposes, and the impoundment of water by man for watering his stock and to supply hydro-mechanical power multiplied the number and extent of breeding places of *Anopheles quadrimaculatus* east of the Great Divide In the far West the mosquito was introduced by man

“The mosquito was introduced by man and the imported descendants of these people were the first to be infected.”

In the middle of the nineteenth century, malaria existed in varying degrees of intensity throughout most of the United States with the exception of the highlands and nonirrigated desert land

Shortly thereafter the disease began to contract away from its more northern limits

La prophylaxie la plus récente et la plus efficace semble être l'emploi du DDT, soit comme lutte anti-imago, soit comme lutte anti larves, soit même comme lutte combinée. Nous avons pu nous même constater dernièrement en Italie et en Sardaigne l'efficacité (du moins temporaire) du DDT sur les anophèles adultes et sur leurs larves.

Mais il y a anophèles et anophèles. Tous ne réagissent pas d'une manière identique au DDT.

En Afrique centrale l'anophèle le plus commun et partant le plus dangereux est *A. gambiae*. Mais il existe des régions où le transmetteur est *A. funestus* et dans d'autres, *A. marshalli* et *an. moucheti*, etc. Quant aux gîtes larvaires, ils sont différents suivant les diverses espèces.

Il faut par conséquent, avant de généraliser l'emploi en grand du DDT en Afrique centrale, procéder à une série d'expériences préliminaires, expériences conduites d'après un programme élaboré d'avance par des personnes expérimentées et compétentes dans la matière.

La place nous manque ici pour énumérer et motiver ces expériences. Nous les avons exposées dans une étude spéciale, actuellement sous presse (7) et qui sera soumise au Congrès en même temps que la présente étude.

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Shortly thereafter the disease began to contract away from its more northern limits Some of the authors cited above have speculated on the cause or causes of this decline without agreeing completely as to its detailed *modus operandi* They concur, however, in the conclusion that it was associated with and was probably due to the collective

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Il faut par conséquent, avant de généraliser l'emploi en grand du DDT, faire des essais de lutte anti imago et anti larves sur les différentes espèces d'anophèles.

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effects of improved socio economic conditions and standards of living. In the northeastern quadrant of the country and in the upper Mississippi Valley, the recession of the disease developed spontaneously and independent of conscious, purposeful efforts directed at malaria prevention. Factors which probably contributed to its expiration were

nutrition, clothing, and medical services. The combined impact of these civilizing influences exerted in conjunction with the relatively unfavorable climate for malaria was associated with a drastic retreat of the disease so that by 1900 endemic malaria was restricted to the southeastern coastal lowlands and the lower Mississippi flood plain with areas of persistent but of less importance in the Central Valley of California and the irrigated sections of New Mexico.

Figure 1 shows the officially reported malaria morbidity and mor-

tration Area of the Bureau of the Census was officially established, Arizona, New Mexico, and Oklahoma had not yet been admitted to the Union. The 14 more highly malarious States of the Southeast<sup>1</sup> and the 16 other States not yet included were brought into the Registration Area at irregular intervals over a period of 23 years ending in 1933. Accordingly, annual rates for the Nation have been based by some workers on the total population of the United States, others have preferred to use the aggregate population of States from which

mortality rate curves are given from evident that the differences are most marked early in the century when the Registration Area did not include many malarious States. The disparity diminishes, and the reliability of rates improves, as far as population data are concerned, with accretions to the Registration Area through 1933. This does not imply that the succeeding data are considered completely trustworthy, they simply lack some of the statistical defects of the previous rates. Both before and after 1933, the information contains inde-

<sup>1</sup> Names and dates of inclusion in the United States registration area: North Carolina 1910, Kentucky and Missouri 1911, Virginia 1912, South Carolina, 1915, Tennessee 1917, Louisiana 1918, Mississippi and Florida, 1919, Georgia 1921, Alabama 1925, Arkansas 1927, Oklahoma 1928, Texas, 1933 (10).

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tection of the early settlers permitted mosquitoes to enter free-  
ly, giving these potential vectors with agreeable shelter and  
abundance of food by night. The infected descendants  
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terminate but probably decreasing amounts of reportorial error and inadequacy

When generous allowance is made for these imperfections, it

exception, by the reported experience of the States concerned (8-11), by the general testimony of residents and malaria control personnel (12) and by special field studies (13, 14) in areas where malaria has been highly prevalent in the past. If this negative slope of malaria

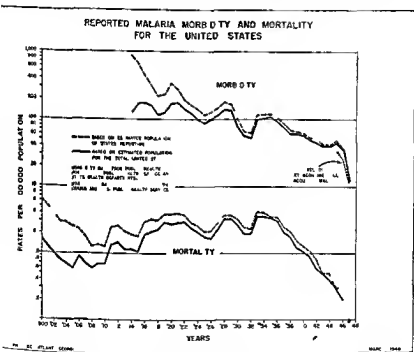


Figure 1

trends can be maintained or accelerated it will mean the ultimate extinction of the disease in this country (15)

Possible factors concerned in this regression have been considered by Andrews (15) and the conclusion is reached that the more important of these are domestic and areal reduction of anophelines by insecticidal, antilarval, and diversionary means, population removal from malarious to nonmalarious areas, and antimalarial medication

Because of the minimal status of malaria, the proposal of Dr L

In figure 3, malaria cases of 10 or less are represented collectively as single dots. For reporting units with more than 10 cases, the morbidity-rate category is shown.<sup>4</sup>

The paucity of the cases in Canada suggests either serious under-reporting or a negligible health problem, probably the latter. Presumably all of the cases were veterans relapsing with vivax infection acquired overseas.

The 1947 malaria morbidity map for the United States shows an

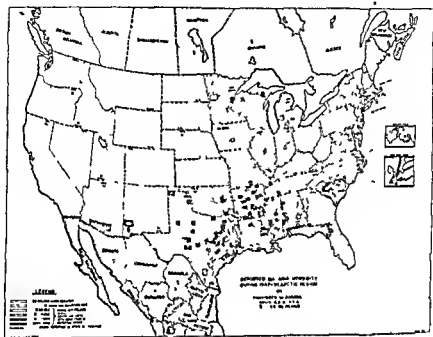


Figure 3

enormous scatter of small numbers of cases throughout the country with heavier concentrations in the Southeastern States. They ag-

... of ... in ... (in parentheses) for the year 1947

and the death rate categories by county (United States) or State (Mexico).<sup>2</sup>

There were no malaria deaths reported in Canada during 1946.<sup>4</sup>

In the United States, the number of deaths certified due to malaria from 35 States and the District of Columbia in 1946 was 341, the lowest for the country since complete statistical coverage has been achieved. The majority of these, 274, occurred in the traditionally paludic States of the southeastern quadrant. Texas had the highest number, 63, but its mortality rate (0.9/100,000) was equaled or exceeded by Alabama, Arkansas, Florida, Louisiana, Mississippi, and South Carolina.

The malaria deaths reported from the remaining States are believed to be due mainly to malaria contracted elsewhere, either abroad or in endemic areas of this country. However, the standard statistical practice of referring all deaths to the counties of residence, irrespective of where they occurred, results in occasional geographic discrepancies between place where malaria was acquired and where death due to malaria supervened. There is also something more than a suspicion that many of the deaths certified as being due to malaria, both within and outside the southeastern quadrant of the country, were actually not caused primarily by malaria. Thus vivax infection, especially when adequate medical services and hospitalization are available and utilized, is rarely a primary cause of death, yet it has been so certified repeatedly during the last 3 years.

The number of malaria deaths and death rates reported from Mexico in 1946 are of conspicuously higher orders of magnitude than for any other portion of the Nearctic Region. This argues either gross over reporting or a much more important health problem due to malaria than has occurred in the United States for many years. Deaths from malaria are less numerous on the highland plateau which occupies the north central part of the country. Their highest concentration is in Tamaulipas and Nuevo Leon, in the eastern coastal plain at the terminal portion of the Rio Grande. The high death rates in the two southernmost counties of Texas, Hidalgo and Cameron, appear to be related to this highly endemic area. Sonora and Sinaloa, Mexico, also have relatively high rates.

<sup>2</sup> Actual number . . .

TABLE 1.—*Malaria morbidity by source for divisions of the United States reported from counties and independent cities reporting cases during 1945, 1946 and 1947<sup>1</sup>*

| States of the United States          | Counties and independent cities |                        |  |                                |                      | Reported cases <sup>2</sup> |                      |                       |                      |
|--------------------------------------|---------------------------------|------------------------|--|--------------------------------|----------------------|-----------------------------|----------------------|-----------------------|----------------------|
|                                      | Total                           | Number reporting cases | Percent of number reporting cases <sup>3</sup> |                                |                      | Total                       | Percent acquired     |                       |                      |
|                                      |                                 |                        | Acquired within United States                  | Acquired outside United States | Source not specified |                             | Within United States | Outside United States | Source not specified |
| Traditionally malarious <sup>4</sup> | 1 445                           | 1,064                  | 52   | 19                             | 29                   | 102,570                     | 82                   | 10                    | 8                    |
| Others                               | 1 652                           | 929                    | 17   | 65                             | 28                   | 24,050                      | 5                    | 84                    | 11                   |
| Total                                | 3,097                           | 1 993                  | 52   | 49                             | 34                   | 126,620                     | 67                   | 21                    | 1                    |

<sup>1</sup> From Public Health Reports, U. S. Public Health Service, and reports from State health departments.<sup>2</sup> Includes some cases not designated from counties and independent cities.<sup>3</sup> Not mutually exclusive categories—counties may report in each class.<sup>4</sup> See footnote 1 in text.

All counties (and independent cities) reporting malaria and all malaria cases reported during 1945, 1946, and 1947 were analyzed with reference to the indicated sources of infection. The counties and the cases were then consolidated by States, and the States by groups according to their historic malariousness. Table 1 shows a summary of these findings.

About two thirds of the counties and independent cities of the Nation reported 126,620 cases of malaria during this triennium. It is, perhaps, not surprising that the traditionally malarious States of the Southeast considered the preponderance of their cases to be indigenous, whereas a comparable majority in the other States was ascribed to extracontinental infection. This was reflected not only in the numbers of counties reporting cases acquired within and outside the United States but also in the numbers of cases recorded from these sources. Returns from the relatively malaria free States showed less variation with respect to overseas infection than did the numbers of the locally acquired cases in the more malarious States, though there were notable departures from consistency in both groups. One South Central State claimed that only 1 percent of its cases were of overseas origin, another, bordering on the Great Lakes, indicated that 27 percent of its malaria case load originated within its borders. Certain States reported all cases of malaria diagnosed within their boundaries during the year, others refrained from reporting obvious relapses because these cases had not been acquired during the current year and had been reported previously.

All told, there is undoubtedly a higher proportion of extracountry cases as shown in table 1. Actually the less country cases and overseas

gregate 16,203 from 46 States and the District of Columbia another all time low since all States were included in the Registration Area. The total for the 14 traditionally malarious States is 14,505, or 90 percent of all cases for the country. Texas leads the country with 4,856 cases reported; South Carolina is a close second with 4,608. Other States with relatively high rates but not so many cases are Arkansas, Mississippi, and Oklahoma.

The less than 2,000 cases reported from the rest of the country are accounted for mainly by relapses of vivax malaria acquired extra continentally, though a few were due to blood transfusions, malaria inoculated for therapeutic purposes, and possibly to blood contamination during parenteral injection of split doses of drugs among addicts. Cases from similar sources also occur in the Southeastern States, though they are more difficult to identify.

The morbidity picture in northern Mexico is essentially similar to that of malaria mortality, higher rates being reported from the coastal

seas service in the tropics, it was evident\* that a substantial amount of malaria morbidity due to recurrences would be recorded during the subsequent years from civilian agencies (including Veterans Administration Facilities) as well as military ones. Unless these imported infections could be distinguished from indigenous malaria, unwarranted inferences might be made concerning the establishment of new and the intensification of old areas of endemicity. Accordingly, the Surgeon General of the Public Health Service requested State health officers reporting malaria cases to furnish information (based on presumptive evidence) whether these were contracted "within or outside the United States" (18). This request was complied with to an indeterminate extent since 1945.

The short, finely broken line in figure 1 marked "excluding extracontinentally acquired malaria" is the morbidity rate curve based on the estimated population of the whole United States for 1945, 1946, and 1947, minus the malaria reported to have been contracted overseas. The difference between the two curves is greatest for 1946, as might be expected because demobilization took place in late 1945 and in 1946. The smaller difference in 1947 probably reflects the tendency of vivax recurrences to diminish in frequency with time. It seems evident that the inclusion of these cases accounts for the irregularity in the graphs of total morbidity for those years, without the extracontinentally acquired malaria, the case rate curves would fall as smoothly as the corresponding death rate curves.

\*According to provisional data furnished by the Medical Statistics Division of the Surgeon General's Office, United States Army, there were some 92,000 admissions for malaria in military hospitals within the continental United States during the quadrennium 1942-45. Most of these were relapses of infections acquired overseas.



## TROPICAL MEDICINE AND MALARIA

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The proportions of the countries and cases included under the caption "Source not specified" showed the greatest variation. This is probably a rough measure of the failure of practicing physicians and health authorities to determine and specify likely sources of infections of cases coming to their attention.

Thus the principal effects of extracontinentally acquired malaria in the Nearctic Region have been (1) to increase the specific morbidity rates for the Nation during and after World War II, and (2) to introduce malaria cases into every State and about two thirds of all the countries in the Nation.

As far as can be ascertained, the presence of infected individuals throughout the country has not resulted in significant transmission of the imported parasites, though their infectiousness to North American species of anophelines has been demonstrated (19, 20). Such a possible consequence was feared and prudently publicized (21, 24) during World War II, but the author is aware of only a single instance in the Northwest in which the probability of malaria transmission from a veteran has been reasonably well established (25). Such dissemination would be expected to occur most readily in the Southeastern States where it would be exceedingly difficult to detect with certainty, perhaps it has taken place more frequently than is known.

Notwithstanding the downward trend of malaria prevalence which existed before 1941, it is a tribute to the mighty preventive efforts of military and civilian agencies that no discernible increase in malaria transmission has occurred during and since the end of World War II.

### SUMMARY

In conclusion the present status of malaria in the Nearctic Region may be summed up as follows:

There appears to be a possibility that malaria may be eradicated from the United States and its possessions within a few years.

In the northern states of Mexico, especially on the coastal plains, malaria remains a perennial and important public health problem.

Thus far, the repatriation of malaria infected American veterans has resulted only in a slight and apparently transient increase in reported morbidity, due primarily to clinical reactivation of cases acquired abroad.

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# THE MALARIA PROBLEM IN THE NEOTROPICAL REGION

ARNOLDO GARILLON, *Director de Malaria en el Marañon,  
Arequiva, Venezuela*

The Neotropical Region is practically the whole territory almost all of it south of the Tropic of Cancer known in geography as Latin America. It has about 10 percent of the dry surface of the earth and 5 percent of its population belong to the White Amerindian and Negro races. Malaria is present in different proportions almost throughout all these lands with the exception of Uruguay, the only country free of it, and Chile, where it has been recently eradicated.

## VECTORS

The complete individuality of the Neotropical Region in respect to the anopheline fauna is remarkable, as has been pointed out by Christophers (1933). It has a genus of its own the genus *Chagasia*, and of the seven generally accepted subgenera of *Anopheles* five are peculiar to it *Arthromyia*, *Lophopodomys*, *Kerteszia*, *Lyssorhynchus* and *Stethomyia*. Furthermore the most prevalent species of the subgenus *Anopheles* found here belong to groups which are peculiar to this zone *Arribasagua*, *Ayrazomyia* and *Shannonimyia*. The number of species present in this region comprises 29 percent of the world anophelines according to the list given by Russell Rozeboom and Stone (1943).

Of the six subgenera of *Anopheles* present in the Neotropical Region three have vectorial species *Anopheles Kerteszia*, and *Lyssorhynchus*. Seventeen species have been found naturally infected with malaria parasites, but on epidemiological grounds the majority can be eliminated as important vectors, although some of them may have considerable local importance in relatively circumscribed districts, probably due to exceptional ecological conditions responsible for unusual high densities of the mosquito populations. Reference, therefore, will be made only to the prominent ones which are *A. pseudopunctipennis* and *A. punctimacula* of the subgenus *Anopheles*, *A. bellator* and *A. guasalis* of the subgenus *Kerteszia*, and *A. albitarsis*, *A. albitarsis*, *A. The distribution of the neotropical anophelines follows closely the* of the generally accepted subregions. *A. albitarsis* the main vector of the Mexican subregion invades the northern portion of the Caribbean subregion and the northwestern part of the Brazilian subregion, especially of the southeast. *A. aquasalis* of the Caribbean region, invades the southern portion of the Antillean. *A. bellator* relatively small districts of high prevalence in the north and

be unique

The vectorial power of the neotropical malaria carriers may be judged from their sporozoite index. In table 2 it may be seen that *A. darlingi* is by far the best carrier, with an index of 0.9, and *A. albimanus* the second best, with 0.6. It is probable that both *A. pseudo punctipennis* and *A. albivittatus* show indexes in this table which do not represent true field conditions in all their distribution areas, as they are complexes of subspecies which may have different regional significance as malaria vectors. The important fact shown in table 2 and in figures 1 and 2 is that they are much weaker carriers than some from the Ethiopian and Oriental Regions, where mosquitoes are found to be much more efficient. This explains the fact that the neotropical authorities of the continent have not yet found an efficient African vector, which has invaded Brazil.

### EPIDEMIOLOGY

The general features of neotropical malaria, as elsewhere, are connected with the vectors present. It has been seen that they are less powerful carriers than those from other tropical territories, a consequence of which is that the incidence of malaria is much lower than in the high degree of endemicity found in the tropics.

The high rates of mortality known from Punjab, India, the difference being possibly influenced by the general low density of the human population found throughout our region.

Of these epidemics, some have a great relationship to increased population density, as was the case in Yucatán in Mexico, and in the state of São Paulo, Brazil, by *A. albimanus* observed in Brazil.

connections

changes in

increase in its population densities and distribution range. These have given rise in Venezuela to a rather regular 5 year cycle in malaria.

..

recorded from  
*manus*, and in the  
*punctipennis*, as  
as high as the

ones known to be due to *A. darlingi*.

The Neotropical Region extends north and south of the tropics,

*A. cruzi* in the south of the Brazilian subregion. *A. darlingi* is distributed throughout the Brazilian subregion and has a small zone where it was probably artificially imported in the Mexican subregion. *A. pseudopunctipennis* is the mosquito with the largest area of dis-

tribution throughout the Mexican subregion and the northwestern portion of the Brazilian and Patagonian subregions.

One peculiar feature of the neotropical anophelines is the altitude they attain. Nowhere else in the world are mosquitoes of this tribe found at such high altitudes (table 1). At least 24 species extend above 1,000 meters, and of these, 5 reach 2,000 meters and 4 the 3,000 meter level (10,000 feet). *A. albimanus* and *A. darlingi* transmitted malaria practically disappears above 1,000 meters, but *A. pseudopunctipennis* produces malaria even at 2,773 meters (9,100 feet), that is, the highest malaria in the world.

TABLE 2—Anopheline vectors of malaria in the Neotropical Region based on data taken from different publications

| Species                      | Countries  | Stomachs       |               |          | Glands         |               |         |
|------------------------------|--|----------------|---------------|----------|----------------|---------------|---------|
|                              |  | Dis-<br>sected | Posi-<br>tive | Index    | Dis-<br>sected | Posi-<br>tive | Index   |
| NEOTROPICAL                  |  |                |               |          |                |               |         |
| <i>A. albimanus</i>          | British Honduras, <sup>1</sup> Costa   | 8 031          | 38            | 0.8±.12  | 6 086          | 35            | 0.6±.10 |
| "                            | " " "  | "              | "             | "        | "              | "             | "       |
| <i>A. alb.</i>               | " " "  | "              | 65            | 1.0±.24  | 1 553          | 4             | 2±.11   |
| <i>A. species</i>            | Brazil, British Guiana, <sup>1</sup> French Guiana, <sup>1</sup> Grenada, <sup>1</sup> St. Lucia, and Trinidad | 4 077          | 102           | 2.5±.24  | 5 070          | 30            | 4±.09   |
| <i>A. sp.</i>                | B. W. I.   | "              | 25            | 6±.13    | 2 145          | 3             | 1±.08   |
| "                            | "  | "              | 27            | 1.2±.23  | 1 008          | 1             | 1±.09   |
| "                            | "  | "              | 279           | 4.4±.28  | 5 082          | 47            | 9±.13   |
| "                            | "  | "              | 64            | 1.3±.18  | 4 985          | 23            | 5±.10   |
| <i>A. pseudopunctipennis</i> | Colo. Brit. Panama, Ecuador, <sup>1</sup> Costa Rica, Peru, Venezuela  | 1 111          | 20            | 1.1±.25  | "              | "             | "       |
| ETHIOPIAN                    |  |                |               |          |                |               |         |
| <i>A. gambiae</i>            | Brazil   | 3 863          | 896           | 23.2±.57 | 4 170          | 256           | 6.1±.37 |

<sup>1</sup> The species was not found naturally infected in this country.

<sup>2</sup> Natural glandular infections reported from Peru but in less than 1,000 dissected specimens.

<sup>3</sup> This species was eradicated in 1949.

The remote taxonomic relationships of the vectorial species probably are responsible for the striking differences they show from behavioristic and ecological standpoints. These, and their genetic con-

spring, which is much smaller than the corresponding one of the north

Another interesting characteristic of the neotropical malarias is



Figure 2.—The geographical distribution of *A. albivittatus*, *A. bellator*, *A. punctimacula*, and *A. cruzii*

the geographical distribution of their parasites (fig 4) *P. falciparum* is more abundant north of the Equator than south of it, in general it decreases from the Caribbean Islands westward and southward *P. malariae* does not show a regular trend in its distribution

latitude having an influence on the seasonal curve of malaria. North of the Equator the seasonal wave reaches its acme in the second half of the year, and south of it in the first half. Near the Equator



Figure 1.—The geographical distribution of *A. albimanus*, *A. darlingi*, *A. aquasalis* and *A. pseudopunctipennis*.

the amplitude of the wave is some times very small, due to few seasonal changes in rainfall, and north and south of it, the dry seasons, by reducing the anopheline population, diminish or interrupt transmission. The seasonal wave in general has a single peak, and only in the southern subtropical zone is a second peak present during the



site to reach the thirty third parallel, the others stopping at the twenty ninth, it produces, therefore, the southernmost malaria known. The distribution of these plasmodia is not only due to geographical conditions, but possibly has also a relationship with the human races present. The high prevalence of *P falciparum* in the West Indies may depend on the predominant Negro element in many of them. *P malariae* has also an incidence in Negroes very different from that in East Indians inhabiting the same islands (Downs, Gillette and Shannon, 1943). Nevertheless, it seems that the present distribution of the malaria parasites cannot be explained only from the standpoint of geography and race.

As the predominant vectorial species in each one of the four neotropical subregions are different, it is desirable to consider other



Figure 4.—The geographical distribution of the malaria parasites in the Neotropical Region

features of the prevalence of malaria separately in each one of the Mexican subregion.—The main vectors are here *A pseudopunctipennis* in the highlands and *A albimanus* in the lowlands. The former species exists in the Nearctic Region, but *A albimanus* appears in the northern boundary of the Neotropical Region in Mexico and being a vector of greater potency than neighboring species, originates an increase in the prevalence of malaria. *A dopunctipennis*, although present throughout this subregion, is not a vector in Mexico and Guatemala, no data at the present time available to explain why it does not produce malaria in the countries. This anopheline is a species complex, which may elude the problem on taxonomical grounds. But as we still lack a universally adapted to measure mosquito densities in survey impossible to discard this population factor as an alternate explanation. In Mexico, for instance, many thousand mosquitoes caught in a short time inside houses (Vargas, Casis, and Earl

Zones where it is relatively frequent alternate with others where it is exceptionally scarce. *P. vivax*, on the other hand, increases south

SPOROZOITE INDEXES OF MAIN VECTORS FROM NEOTROPICAL  
ORIENTAL AND ETHIOPIAN REGIONS

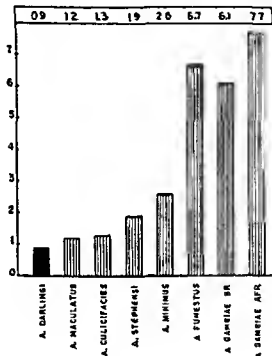


Figure 3.—To compare the sporozoite index of *A. darlingi* with those of some Oriental and Ethiopian species. The indexes of *A. gambiae* are from Brand and Africa.

ward and with height. It is the only species found at 2,773 meters (9,100 feet) where the highest malaria of the world has been reported (Moscoso Carrasco, 1943), although the other two have been observed at 2,440 meters (7,990 feet). *P. vivax* is also the only para

incidence of malaria in this country should be connected with the low prevailing per capita income, which appears to be the only existing difference from other people in nearby areas. This would then be a clear example of low economic conditions helping the prevalence of the disease. In the Lesser Antilles, the islands where *A. aquasalis* is the only vector, malaria is of lower incidence than in the northern ones where *A. albimanus* is also found.

**Brazilian subregion**—Of the four neotropical subregions, this is the only one that extends north and south of the equator. It has all the important known vectors of the region, five of them peculiar to it. Malaria prevails at a level of widespread intensity unknown in the other subregions, and its incidence seems to be intimately connected with the vectorial species present in a given district. *A. darlingi* transmitted malaria is found in general at an intensity unusual in

#### subregions

Spleen rates above 50 percent are not uncommon, but the very high ones above 80 are a rarity, and are found usually in small villages. This means that malaria does not reach here the high level of endemicity which is known in other tropical portions of the world, which may be understood if it is remembered that *A. darlingi*, the most potent neotropical vector, has a sporozoite index 12 to 18 times smaller than those found in some Oriental and Ethiopian species. This is very important, because frequently the intensity of malaria is presented in the usual textbooks as if it were similar throughout the equatorial climates.

North of the Equator malaria seems to be of higher intensity than south of it. It is also not so prevalent on the Equator itself, in the Amazon Valley, as it is in the nonequatorial climates of the north. This is probably due to the fact that *A. darlingi* does not reach the high densities found in the northern zones, as it has been observed that in the black water rivers of this basin, where acidity is high, this species is absent, and, therefore, no malaria is present (Gabaldon, 1948). Also, a similar absence of malaria due to ecological difficulties found by this mosquito exists in some districts of the Venezuelan savannas, where marked dry and wet seasons are present.

**Patagonian subregion**—Of all the neotropical subregions, this is the one with the least malaria. *A. pseudopunctipennis* is the only vector, with the exception of small districts in the northwest, where *A. punctimacula* is also a carrier. In the Peruvian coastal valleys spleen indexes from 10 to 25 percent have been found (Paz Soldán, 1934, Quirós Salinas, 1943), but in Bolivia higher indexes have been recorded, some reaching even 95 percent (Moscoso Carrasco, 1943). In Chile, the only neotropical country where malaria appears to have been entirely eradicated through a well carried out campaign

which certainly is not the case in Venezuela where *A. pseudopunctipennis* plays practically no role in transmission.

In Mexico malaria is more prevalent on the Gulf than on the Pacific side, as *A. albimanus* has a wider distribution in the former. In the southern states of this country, however, the disease reaches higher intensities on the Pacific. In some portions of the Yucatan Peninsula, with low rainfall, malaria is as low as in the nearctic regions of Mexico, in spite of *A. albimanus* being the vector. Here, epidemics have been reported in years of exceptional precipitation.

From Mexico southwards, with some local exceptions, Pacific side malaria is in general similar in intensity to that on the Atlantic side, with the possible exception of Costa Rica and Panama, where the disease seems to be of higher prevalence on the Pacific side. Of all the Central American countries Costa Rica is the least malarious, the

meters, with the exception of Mexico and Guatemala, where highland

most of them of continental origin, with the exception of the Bahamas where malaria is not present, *A. albimanus*, extending its range from

meters in the subregion, the disease being, therefore, more prevalent near the coast.

Cuba and Jamaica, the nearest islands to the Mexican subregion, show a much lower incidence of malaria than the nearby continental countries. In Cuba, for instance, only 8 of 134 municipalities were found with spleen indexes above 20 percent (Carr and Hill, 1942), and in Jamaica

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Rico being 23 percent (Earle, 1930). Rainfall cannot explain the unusually high prevalence of malaria in Haiti, and it seems that exceptional topographical features or agricultural enterprises favoring extraordinary production of *A. albimanus* are also lacking. Then, if an explanation from the entomological side cannot be found, the high

seems to exist a zone of "anophelism without malaria" in the Andean zone of the Patagonian subregion. This fact may depend on the low temperature present at such heights, but as the highest level of malaria is variable in different zones, it may also depend on other factors, such as density of the anopheline or human populations.

### CONTROL

Probably the best and longest experiment on malaria control through drugs has been carried out in the Neotropical Region, in Panama (Clark and Komp, 1941). After 10 years the conclusion was reached that with quinine, quinacrine, and plasmochin, it was impossible to reduce malaria in a zone with abundant vectors. Treatments in Venezuela given without medical supervision were unable to reduce malaria mortality in epidemics (Gabaldon, 1948). Administra-

ratio has and traction, h cations potential ties, it is not believed that under neotropical conditions much can be expected from malaria chemoprophylaxis.

Reduction of anopheline vectors was proved for the first time to be

sion, which makes practically impossible the lowering of mosquito densities at a reasonable cost. With *A. pseudopunctipennis*, however, a decrease in malaria has been obtained through oiling and paris greening in Argentina, Bolivia and Peru. Drainage and filling have given very good results with *A. albimanus* in the Mexican and Antillean subregions, and with *A. darlingi* and other species in some parts of South America. The development of paved drainage ditches in the Neotropical Region has reached a technical stage practically un-

of adult mosquitoes inside the house is a method born in the Neotropical Region 40 years ago (Chagas 1908). It has been replaced today by residual spraying with DDT, which has been found effective against most of the neotropical vectors. Residual spraying not only produces the interception of the infected vectors, but actually

(Neghme, 1947 p. c), it was of importance in some villages of the western slope of the Andes. In Bolivia and Chile *A. pseudopunctipennis* produced only highland malaria, but in Argentina it reaches the flatlands of the northwestern pampas, where the disease is moderately endemic. In this zone, topographical conditions are suitable for large scale production of this anopheline, but the succession of seasons is very unfavorable since it is mainly a stream breeder, the rather dry winter is followed by heavy rains in the summer, leaving only two widely separated periods of 2 months each for uninterrupted breeding. Were it not for this, malaria would undoubtedly be intense, as is shown by the severity of the occasional epidemic years when a mild winter is followed by a summer of light and well distributed rainfall (Hackett 1945).

quate breeding conditions for *A. pseudopunctipennis*, malaria is a highland disease, and does not go south of the tropics only reaching valleys between the 20° and 21° parallels. On the eastern slope of the Andes malaria is found at lower levels and goes as far south as the 33° parallel. Nowhere else in the world does malaria reach this latitude. In both zones, temperature limits the southern distribution of the vector. On the Chilean side, altitude lowers the temperature to an unsuitable degree while still in the tropical belt. On the Argentine side, the altitude being much less, it does not become cold enough till 13° to the south.

In the Andean valleys of Bolivia and Peru *A. pseudopunctipennis* reaches heights unknown for any other anopheline vector. In Bolivia the highest malaria of the world has been reported from Colchia at 2,773 meters (9,100 ft.), and an epidemic with 500 cases has been observed at Cochabamba at 2,600 meters (Hackett, 1945). In Argentina a small epidemic has been observed at 2,180 meters. In Peru endemicity is found at 2,080 meters and in Chile a spleen index of 60.8 percent was recorded at 1,980 meters.

In the other neotropical subregions with few exceptions malaria does not reach such high altitudes. In the places where malaria is known to occur above 1 000 meters it is almost always connected with

Colombia and Peru

*macula* which pro

Colombia Above

in Ecuador at 2400 meters

Guatemala at 2 000 meters

meters (Hoffman, 1937)

In the Antillean and the Brazilian subregions, with the exception of Colombia and Ecuador, malaria is not known above 1,000 meters.

From these data it may be observed that malaria does not reach the same altitude that *A. pseudopunctipennis* does, and, therefore, there

ing interest in malaria control in these republics is shown by the fact that the national budgets devoted to its control have increased four fold in these last 7 years. The international malaria courses of 4½ months duration held in Venezuela, the fifth to be given this year,

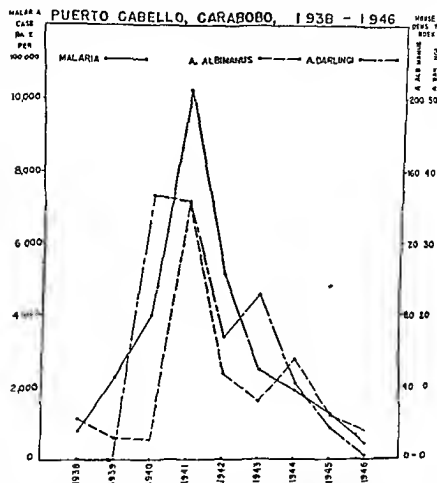


Figure 6.—The morbidity rates shown in figure 5 accompanied by the house density indexes of *A. darlingi* and *A. albimanus* to show that the increase in morbidity is due to cyclical increases of mosquito densities

nable the student "to undertake any kind of work which relates to malaria" (C Wilcocks, Tropical Diseases Bulletin 1945). The recent budgetary increase of the Pan American Sanitary Bureau will permit enlargement of its anti malaria activities, and will speed up the action against the disease. The work of one of its committees, the Pan American Malaria Commission, has contributed much in the standardization and generalization of the most efficient and economical methods

effects a reduction of the anopheline populations by decreasing the

cies which possibly may be eradicated from limited zones through DDT residual spray as the only measure. The *Kerteszia* again can not be affected by this method as they do not rest inside houses. This level it has in the Neotropical Region. For instance, according to

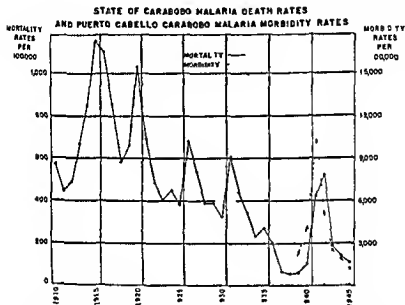


Figure 5.—The 5-year cycle of malaria in Venezuela as shown by death rates since 1910 in the State of Carabobo. The last peak is accompanied by morbidity rates from one town of same State obtained by weekly house to house visits in search of fever cases to take slides for microscopical diagnosis.

some outstanding malarialogists who have personally seen the work, the nation wide campaign carried out in Venezuela at the present time may make this country in the near future the first tropical country to eliminate malaria as a public health problem (table 3 and figs 5 and 6).

The neotropical national governments have an average annual per capita expenditure of \$16 as compared with less than \$5 in the other tropical regions of the world, and as more money for control is spent than in other countries with similar or worse malaria problems, a greater decrease of the disease should be expected here. The awoken



## ABSTRACT OF DISCUSSION

Dr ALVARADO (Argentina) I want to add only a few words to the excellent paper of Dr Gabaldon

No one discusses the new insecticides that are so powerful and active as larvicides and mosquitocides, that DDT at the rate of

malariologists are divided into two groups. First, those who believe that DDT has brought a tremendous improvement and a revolutionary change in techniques and methods in malaria control and its economical basis. Second, those who make some objections, especially about three points: (1) danger of a break down of immunity in a hyperimmune population, (2) the failure of residual sprays against mosquitoes biting outdoors, (3) the low standard of life of some native population incapable of supporting the cost of malaria control measures.

I shall try to answer these three points. (1) The danger of a break down of the immunity in the hyperimmune population. Hyperimmunity is a consequence of hyperendemicity. If hyperimmunity is

infection, malar antimalarial work, we have now wonderful and cheap drugs for controlling mortality and morbidity.

(2) The failure of residual sprays against mosquitoes biting outdoors. These mosquitoes are rather limited to some regions, or are especially secondary vectors effective when the carriers of gametocytes are above some critical level maintained by domestic vectors.

We do not know much about the special conditions in which these mosquitoes transmit malaria, but allow me to say, please try DDT, and try, and we shall see what happens.

(3) The low standard of life for supporting the cost of control. Doubtless, without money nothing can be done, but the tropics are potentially rich. One dollar spent on malaria control means a lot of much more than one dollar almost immediately recovered. New insecticides will be the spearhead for the conquest of the tropics. Malaria must disappear from every civilized country of the world. With the aid of new insecticides, noncivilized countries will be made sooner civilized and, as a natural consequence, malaria free. It is our faith and our pledge.

Dr VISWANATHAN (India) I was interested in the observation made by Dr Gabaldon that in Venezuela anophelines with high anthropophil

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, 60 to 90,

of control. It may be expected that this Commission will greatly help the work of the Expert Malaria Committee of the World Health Organization in the Western Hemisphere. Finally, I may say that the

remarkable

TABLE 3--*Relationship between malaria morbidity rates and density of anopheline vectors in a quinquennial cycle in the town of Puerto Cabello, Carabobo, and malaria death rates in the State of Carabobo, Venezuela*

| Year | Malaria case rates per 100,000 | Specific density indexes (houses) |                    | Malaria death rates per 100,000 in the State of Carabobo |
|------|--------------------------------|-----------------------------------|--------------------|--|
|      |                                | <i>A. albimanus</i>               | <i>A. de Longi</i> |  |
| 1935 | 803.8                          | 23.2                              |                    | 49.2   |
| 1936 | 2,312.1                        | 12.7                              |                    | 58.8   |
| 1940 | 4,083.2                        | 11.9                              | 86.9               | 100.3  |
| 1941 | 10,267.3                       | 145.3                             | 36.0               | 422.8  |
| 1942 | 5,199.3                        | 48.3                              | 17.1               | 551.2  |
| 1943 | 2,500.5                        | 32.5                              | 23.2               | 190.8  |
| 1944 | 1,063.5                        | 56.1                              | 10.6               | 140.4  |
| 1945 | 1,291.3                        | 23.2                              | 4.4                | 109.4  |
| 1946 | 413.5                          | 16.8                              | 5                  | 83.9   |

Mosquitoes captured per 100 house visits

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trations (0.5 percent aqueous solutions), in order to achieve the eradication of *Anopheles pseudopunctipennis*. Aircraft, automobiles and trains were also sprayed every month, especially those coming from

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tiv

Strict checking of *Anopheles* still continues. In order to protect

malaria control from its southern valleys

and A. Neghme

Dr. JUAN MONTALVAN (Ecuador). A la magnífica exposición hecha por el Dr. Gabaldon, solo quiero expresar mi aplauso y añadir unas pocas informaciones respecto del paludismo en mi país, la República del Ecuador.

El hecho de estar la República del Ecuador atravesada por la línea equinoccial, hace que se encuentra dentro de la zona del más completo dominio del paludismo. Pero como ustedes podrán observar en la lámina proyectada, el país está atravesado por la Cordillera de los Andes que se divide en dos cadenas longitudinales, entre las cuales queda una gran meseta (de 2000-3000 m. de elevación) donde el paludismo no es usual, sino en los valles más profundos formados por los ríos que, naciendo en las cordilleras se dirigen hacia las zonas bajas del país.

A uno y otro lado de las cordilleras quedan zonas donde el clima es de lo más favorable al desarrollo del paludismo. Considerado así el aspecto geográfico, tenemos en Ecuador una zona occidental baja de gran endemicidad, donde el paludismo abarca una muy extensa región. En esta zona la transmisión es hecha por *A. albimanus* del cual se forman abundantísimos criaderos durante la estación lluviosa, pero desapareciendo casi totalmente en la estación seca, durante la cual apenas quedan criaderos en zonas muy bajas.

En la región interandina el transmisor es *A. pseudopunctipennis*, cuyos criaderos se mantienen generalmente por todo el año pues se forman especialmente en las laderas de los ríos y en aguas utilizadas para regar. Aquí hay zonas de muy elevada incidencia pues además el *A. pseudopunctipennis* se ha manifestado un magnífico transmisor.

En la región oriental (poco explorada) la malaria se manifiesta en

dosage of 60 milligrams per square foot we have not observed any reduction in larval density of this species. Perhaps it is due to our small dosage or to the outdoor resting habit of the species. With application of DDT as adopted by us, perhaps the species live long enough to breed but not long enough to transmit.

Dr A. NEGhme (Chile). We must point out the results of the anti-

Lluta, Azapa, Vitor, Camarones, Pica and several others of minor importance (from 18° to 21.5° S). Malaria morbidity ranged from 50 to 100 percent of the inhabitants of endemic zones, measured by the splenic index. The parasite index, measured after several standard examinations, ranged from 19.27 to 40.5 percent.

*Anopheles pseudopunctipennis* was the only species present, and it bred from the coast up to 2180 meters of altitude. Its breeding places were well lighted streams rich in algae, shallow pools with gently moving water, puddles and artificial containers. The sporozoite index was 1 percent and the oocyst index ranged from 0.91 to 2.3 percent. In 1937, the antimalarial campaign was begun, under the supervision of Professor Noe first in Arica city and the near Azapa Valley, in 1941 it was extended to the Lluta valley and afterwards, gradually, to all the other endemic zones of the Tarapaca Province.

In the beginning, until 1944, the control measures were:

(1) Administration of antimalarial drugs to patients isolated in hospitals or dispensaries, standard curative treatment during the spring to the populations continuously resident in the malarious zone, suppressive treatments.

(2) Antilarval  
rectifications of

bichloro diphenyl tri-  
malarial campaign in  
emeral industries to  
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facture

The control measures were directed chiefly against adult mosquitoes, spraying DDT every three months in the interiors of houses and dependencies, in weak solutions, approximately 0.1 to 0.5 percent in kerosene, water soluble fruit oils and xylol triton X100 emulsions. From 1947 on, DDT was sprayed in concentrations of 1 or 2 percent.

also applied over domestic mammals, every 15 days, in low concen-

# MALARIA PROBLEMS IN THE ORIENTAL AND AUSTRALIAN REGIONS

SIR GORDON COVELL, M D, D P. H, *Director, Malaria Laboratory, Horton Hospital, Epsom, England; late Director, Malaria Institute of India*

The area covered by these two regions extends from Baluchistan in Northwest India to the Marquesas in the South Pacific, a distance of approximately 10,000 miles. For the purpose of this review, it is convenient to divide it into four subregions, as follows

- (1) The Indian subcontinent (excluding Assam) and Ceylon
- (2) Assam, Burma, South China, Formosa, Siam, and Indo-China.
- (3) Andaman and Nicobar Islands, Malaya, Philippine Islands,

Malaria constitutes a major health problem in every country from India to the Solomon Islands and New Hebrides, the Pacific islands north, south, and east of these groups being malaria free

More than 100 species or subspecies of *Anopheles* have been recorded in these regions, but the majority of these take no part in the transmission of malaria. Only about 1 dozen are generally regarded as vectors of major importance, whilst some 16 others are of local importance in certain areas. The spheres of influence of the principal malarial carriers are shown in maps 1 and 2. It should be clearly

areas in which each is considered to be the predominant

## INDIA AND CEYLON

### INDIA<sup>1</sup>

*Regions of high altitude*—Generally speaking, areas situated at

*Foot-hill regions*—of the Himalayan Ranges up to 4,500 feet above sea level, the skirts of the great central plateau of peninsular India on both sides,

<sup>1</sup> In this review the recent partition of the Indian subcontinent into the Dominions of India and Pakistan has been ignored the whole area being treated as a single entity

brotos epidémicos, mas o menos circunscritos, lo que nos hace pensar que no este muy diseminada. Hasta ahora solo hemos encontrado *A rangeli* y es de hacer notar que los valles interandinos que dan hacia esta region no han sido aun infectados.

En cuanto a anofelinos, debemos expresar que además de *A pseudopunctipennis* y *A albimanus* han sido encontrados por nosotros *A rangeli*, *A eiseni*, *A punctimacula*, *A maculipalpus*, *A trianulatus*, otros trabajadores han reportado *A (K) boliviensis*, *A nervali*, *A aquasalis*, y *Chagasia bathanusi*.

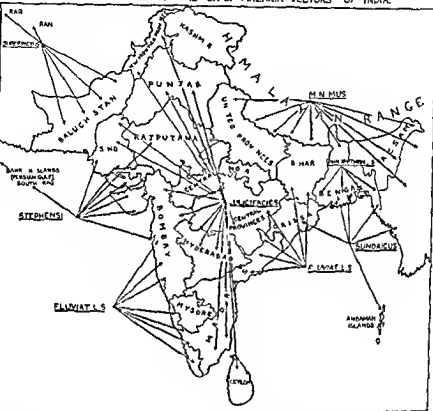
*A pseudopunctipennis*, especie dominante en los valles interandinos ha sido hallada hasta 2560 m y *A albimanus*, dominante en las zonas bajas, lo hemos encontrado hasta 1600 m lo cual constituya una sorpresa para nosotros pues que antes solo habíamos tenido esta especie hasta 300 m.

Ha sido relativamente facil la erradicación de *A pseudopunctipennis* de los valles altos donde se ha trabajado, pero no lo es en zonas más bajas y no ha sido hasta ahora compatible con nuestras posibilidades economicas la lucha contra *A albimanus* en las zonas bajas.—La adopción del DDT nos ha dado, como a muchos, la esperanza de controlar la enfermedad y hemos experimentado tanto con larvicida como adulticida prefiriendo esta ultima en solucion en querosene al 5 percent en aplicación a toda la casa, con lo cual hemos, además conseguido un exito adicional de erradicacion de *A aegypti* en las poblaciones tratados.

*A. philippinensis* as the chief malaria carrier, *A. aconitus* playing a subsidiary vectorial role

*The coastal belt*—Along the western seaboard of peninsular India, there is a strip of low lying land often only a few miles in width, which is almost entirely free from malaria, in striking contrast with

SPHERE OF INFLUENCE OF THE CHIEF MALARIA VECTORS OF INDIA.



the intensely malarious foothills skirting the central plateau. In the coastal zone north of this, where *A. stephensi* is the chief carrier, the degree of endemic malaria varies considerably, being usually mod-

caused by *A. stephensi* occur from time to time. Further north, the picture is complicated by the incursion of the brickish water breeder, *A. sundacus*, which is not found on the western coast. The natural habitat of this species is the estuarine region of Bengal, where it breeds in areas where the mangrove forest has been cleared and the

and the ranges which traverse the northern section of this plateau from east to west

These regions are invariably characterized by the prevalence of hyperendemic malaria, associated with blackwater fever and other so-called pernicious types of the disease. The aboriginal tribes inhabiting such tracts possess a high degree of malarial immunity. They suffer from a universal infection during childhood, but those who survive to adolescence show very little evidence of its effects. Nonimmune immigrants, however, are liable to be stricken down with the intensely severe manifestations of malaria cited.

The principal malaria carriers of the foothill regions of India are *A. minimus* in the northeast and *A. fluvialis* in the south and west. Both these species breed chiefly in stream beds and seepages, and both have a marked preference for human blood.

*The plains*—The vast plains of northwest and peninsular India, extending from the Punjab in the north to Mysore State in the south, are characterized by a type of malaria which is markedly seasonal, with a low or moderate endemicity except where special conditions favour its enhancement.

usually in a season of exceptionally heavy rainfall accompanied by river flooding, following one or more years in which rainfall has been in defect. The effect of such an epidemic on the social and economic life of the community may be so great as to cause serious dislocation of industry, particularly in respect to agricultural activities.

The mosquito vector concerned in the production of these epidemics is *A. culicifacies*, the anthropophilic index of which is normally low.

as an easily accessible food reservoir

*Deltaic regions*—Malaria conditions in these areas differ markedly from those described. As a general rule, endemic malaria is of low or moderate intensity in areas subject to unobstructed and extensive flooding. On the other hand, tracts in which the rivers have decayed, owing to the deposition of silt in their upper reaches and the erection of embankments to prevent flooding or to confine them to a particular course, are usually highly malarious. In striking contrast with the conditions obtaining in northwest India, there is little malaria in areas with a very high subsoil water level, whereas where the water table is comparatively low, the reverse is the case. The principal malaria vector in this area is *A. philippinensis*, a species which breeds in ponds, pools, and ditches containing abundant subaqueous vegetation. In the deltaic region of Orissa, *A. annularis* takes the place of



# ASSAM, BURMA, SOUTH CHINA, FORMOSA, SIAM, AND INDO-CHINA

**Foothill tracts**—This type of country covers the greater part of Assam and practically the whole of north Burma, extending southwards on either side of the Irrawaddy Basin through Arakan on the west and Tenasserim on the east. Similar terrain is found throughout South China, Siam, and Indo China, except in the deltaic regions formed by the great rivers. Malarial conditions throughout these tracts resemble very closely those already described in the Indian foothills. There is the same relative immunity to the disease among the aboriginal tribes, and the same intensity of infections among nonimmune immigrants, associated, as in India, with the prevalence of pernicious forms of *falciparum* malaria and blackwater fever.

The chief malaria carrier of the foothill tracts is *A. minimus*, whose sphere of influence covers a belt of country stretching from the eastern Himalayas to Formosa. *A. jeyporiensis* var. *candidiensis*, which breeds in similar situations, is a vector of some importance in certain areas. *A. leucosphyrus*, a forest species, has been incriminated as a carrier of malaria at Digboi in eastern Assam and was found infected on a number of occasions during the recent military operations in north Burma. Infections have also been recorded in *A. culicifacies* along the Ledo Road, and in certain other localities in north Burma and Indo China.

**Deltaic regions**—Throughout the deltas of the great rivers, endemic

the seaboard of the sea, depending on the presence or absence of suitable breeding places for the brackish water breeder, *A. sundanicus*. Certain tracts are always highly malarious, whilst in others epidemics occur from time to time when local conditions become especially favorable. The coastal belt in China is comparatively malaria free, except where the foothills approach closely to the sea. There are no records of *A. sundanicus* in that country, except that relating to a single specimen captured on the island of Hainan.

# ANDAMAN AND NICOBAR ISLANDS, MALAYA, PHILIPPINE ISLANDS AND EAST INDIAN ARCHIPELAGO WEST OF THE MOLUCCAS AND DAMAR

## ANDAMAN AND NICOBAR ISLANDS

Malaria in these islands is transmitted by *A. sundanicus*, which is the sole vector. It is therefore limited to localities in the vicinity of salt water swamps, or where embankments have been constructed to protect rice fields from the ingress of the tides. Villages within half a mile from such breeding areas are invariably intensely malarious, and outbreaks occur from time to time at distances as far as 1½ miles

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There  
ordering  
the eastern margin of the Chilka Lake on the coast of Orissa. Re

*Urban malaria*—There is only one anopheline vector in India

a number of other urban centres. Outbreaks of malaria due to its presence sometimes assume serious proportions even in the largest cities of India.

#### CEYLON

Malaria in Ceylon is confined to areas below 3,000 feet in altitude. Its dominant feature is the occurrence of regional epidemics at in

The great Ceylon epidemic of 1934-35, the most disastrous in the recorded history of the island, was responsible for 87,000 deaths in the space of 7 months.

Only one anopheline has been incriminated as a vector of malaria in Ceylon, namely, *A. culicifacies*, the same species which is concerned in the production of regional epidemics in India. But, whereas in northwest India such epidemics are confined to years of excessive rainfall and flooding following a period of defective precipitation, the reverse is the case in Ceylon, where they are invariably associated with periods of drought caused by failure of the monsoon rains. The explanation of this apparent anomaly is simple, for in both cases the fundamental requirements for the production of a regional epidemic are fulfilled, namely, the establishment of conditions exceptionally favorable for the production and longevity of the carrier species of Anopheles in the presence of a low degree of communal immunity against malaria. Such conditions are produced in northwest India on the recession of the flood water, whereby innumerable water collections favorable for the breeding of *A. culicifacies* are created. In Ceylon, in years of normal or excessive rainfall, the rivers run full, and breeding places suitable for *A. culicifacies* are comparatively few. In years of defective rainfall, however, the rivers are reduced to sluggish streams with numerous embayments and pools along their course, in which this species breeds in countless numbers.

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*Deltaic regions*—Throughout the deltas of the great rivers, endemic malaria is generally slight or moderate in amount, the vector being one or other of the varieties of *A. hyrcanus*.

*The coastal belt*—The incidence of malaria along the seaboard of Burma, Siam, and Indo China shows wide variations, depending on the presence or absence of suitable breeding places for the brackish water breeder, *A. sundaci*. Certain tracts are always highly malarious, whilst in others epidemics occur from time to time when local conditions become especially favorable. The coastal belt in China is comparatively malaria free, except where the foothills approach closely to the sea. There are no records of *A. sundaci* in that country, except that relating to a single specimen captured on the island of Hainan.

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from any brackish water Cerebral malaria and blackwater fever occur among nonimmune immigrants

## MALAYA

As in India, the hill tracts of Malaya are highly malarious but *A. maculatus* takes the place of *A. minimus* as the principal malaria carrier *A. barbirostris* has been incriminated as a vector of some importance in the plains in certain areas In the coastal belt malaria is absent or moderate in amount wherever the mangrove forest which is especially abundant on the western seaboard has been allowed to remain in its natural condition Where the forest has been cleared and the flow of the daily tides impeded by embankments, roads etc., this zone may become intensely malarious *A. sundaicus* being the vector

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## EAST INDIAN ARCHIPELAGO

In these islands the incidence of malaria is particularly severe in the coastal districts, where the principal vector is *A. sundaicus* The hill tracts are often highly malarious the chief carrier being *A. maculatus*, whilst *A. minimus* var *flavirostris* is said to be of local importance in west Java The inland plains and those lying between the coastal belt and the foothills are as a rule comparatively healthy, but certain areas, such as the marshes of south Sumatra are malarious Here, the chief carrier is one or other of the varieties of *A. hyrcanus* *A. aconitus* also plays a part in the transmission of malaria in the plains, and in parts of west Java this species is considered to be almost as dangerous a vector as *A. sundaicus* In Celebes a high natural infection rate has been recorded in *A. barbirostris* var *tana*

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have been recorded as infected in Borneo are *A. sundaicus*, *A. maculatus* and *A. minimus* var *flavirostris*

## PHILIPPINE ISLANDS

Malaria in the Philippines has never been a problem of overwhelming importance Its highest prevalence is among the foothills,

# MALARIA AS A PROBLEM FOR THE WORLD

## HEALTH ORGANIZATION

E J PAMPANA, M D, *of the Secretariat of the Interim Commission of the World Health Organization, former Secretary of the Malaria Commission of the League of Nations, Geneva, Switzerland*

This Fourth International Congress on Malaria is the first in which the WHO is represented. Within a few weeks the First World Health Assembly will meet, and I have no doubt that it will take aggressive action against the problem that interests us all here.

The fact that the Interim Commission of the WHO has recommended to the First Health Assembly that high priority be given to malaria means that malaria represents an international public health problem. But why is it an international problem?

At first glance, malaria does not appear to have an international character at all, one would almost say that no other disease is so strictly dependent on local conditions. Malaria might, in fact, almost be called a nationalistic, or better still, a localistic disease, because it takes from the country its very characteristics, as does its folklore. But if malariology claims to be a science like any other science, it must cover all the aspects of the problem in its field, it must collect all existing data, classify them, deduce laws and rules, and beware of generalizations. Owing to the different epidemiological constellations that malaria shows in every country, no malariology is conceivable unless it embraces all malarious phenomena, in whatever country they occur. One could imagine that the whole science of venereology might have developed in a single country, but it would be sheer nonsense to believe that malariology could have been brought to its present state of development in a single country, be it the country of Laveran, the India of Ronald Ross, or the classical lands of the Mediterranean where malaria was already described some 24 centuries ago.

Granting that malaria epidemiology should be studied in all malarious countries, one might object, however, that epidemiology is only a part of malariology. Certainly the pathology of malaria does not show such variations in the different countries, but on the other hand, is not control strictly bound up with epidemiology? The history of malaria is, alas, too rich in tragic mistakes made by men who, although well trained and fully conversant with malaria in their own country, believed their knowledge was applicable beyond its borders.

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high natural infection rate has been recorded in *A. barbirostris* var *tana*

In Borneo, the forest species *A. leucosphyrus* has recently been incriminated as the principal vector It seems possible that this species, which, as noted, has been found infected in a number of localities in eastern Assam and north Burma, may prove to be of greater importance in the transmission of malaria in other parts of the oriental region than has hitherto been suspected It has a marked preference for human blood, but since it does not remain in human dwellings after feeding, its presence is easily overlooked Other species which have been recorded as infected in Borneo are *A. sundaicus*, *A. maculatus* and *A. minimus* var *flavirostris*

## PHILIPPINE ISLANDS

Malaria in the Philippines has never been a problem of overwhelming importance Its highest prevalence is among the foothills,

guards the typical, self supporting, rural communities, what could the conference recommend, except extending the free distribution of cinchona products, enlisting the aid of the people in minor control methods, and exploring cheaper methods of control which use time more than money? It then required a great dose of optimism to believe that malaria could thus be reduced. But what else could they have done?

Today, the outlook is entirely changed. We cannot state that residual spraying will stop malaria in all countries, but we do know that, for the time being, this aim has been achieved in some. The law stated above has been broken: it does not cost any more now to protect a hundred people in a village than a hundred people in a town. The aid offered by the people of the villages, to which the Bandoeng conference alluded, can more easily be enlisted for residual spraying than for naturalistic measures, because the people realize the immediate benefits of spraying. If, as it seems, methods are

morbidity, but also, in regard to agricultural countries, of increasing the world's food supply. We know that some Governments have already been able to set up a plan of control, or even of eradication with the available modern methods. Others will need help, and, if they request it, the WHO should be able to grant it.

The assistance to governments can, of course, assume different forms. Even stimulation and coordination of research and the diffusion of information are means of indirect assistance, but a more direct cooperation with governments could probably be carried out by other methods which will be chosen according to needs. There are countries where the Public Health Service is already well organized, and where there is already a will to control malaria, in these cases the need might consist in reorganization of the malaria service, or in a careful survey of the malaria situation and the drafting of a rational plan of control. It would then be sufficient to lend to the

at the appropriate time. Else  
training some malariologists or  
research or of control. This  
might be met either by granting fellowships abroad or by lending experts to carry out the training, for instance, in imago or larval control, to the country requesting it.

But there are countries where malaria is a serious problem, countries which are not yet technically organized to carry out a large scale program of control, or which are not convinced that malaria can be controlled in their particular circumstances, within their financial possibilities. The best means to help would be to send a team which, after making the necessary survey, could carry out control in a selected area of the country. This team should be able to show that







mortality from insect borne diseases other than malaria. The purpose of the teams should be exactly that of giving a demonstration showing (1) how malaria can be controlled, (2) the cost of such control, and (3) what public health improvements and economic benefits would ensue. We do hope that malaria will one day be eradicated from the world, even if that means a deliberate hara kiri for malariology. The problem for the WHO will not be to carry out this eradication, nor even to control malaria all over the world but it will be to rouse public opinion and governments and to let them follow and extend the work once this has been proved possible and successful.

This approach, of course, is not new. Outstanding malarialogists have endeavored to assess the economic damages caused by malaria and, on fewer occasions, to calculate the economic advantages of malaria control. At the Amsterdam Congress, the chairman of the Malaria Commission of the League of Nations stated that such investigations were going to be taken up by the Commission. However, results were somehow not dramatic enough to rouse public opinion. Today, we are in a better situation. Residual spraying alone by its immediate effects on house hunting insects (much more, of course, than by its effect on malaria transmission), so impresses popular opinion that the people themselves demand it again. At this stage, if reliable figures can be produced on the economic implications and results of malaria control, public opinion will support statistical evidence, and governments will have to maintain and extend the campaign. On the other hand, thanks to the popularity of house spraying methods, governments may find a way of reducing expenses by enlisting the aid of the people. This aid, suggested by the Bandoeng conf.

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was another public health measure that could sell so well as residual spraying.

So far, we have indicated two main aspects that the malaria problem presents to the WHO: indirect and direct aid to Governments to combat malaria. There is another aspect, not bilateral, but multilateral, to consider, that is, international protection against the introduction of malaria vectors. Large scale anopheles eradication campaigns

program I tried to develop the program along the lines of the suggestions offered

It has been a matter of considerable regret to me that we did not have more time at our disposal and had very little opportunity to accept papers that were proffered for presentation. We have made use of such proffered contributions insofar as space permitted.

In 1938, during my presidency of the American Society of Tropical Medicine, in my presidential address I discussed malaria in retrospect and prospect. During that address I expressed the opinion that the control of malaria, as observed up to that time, had probably progressed about as far as might be expected from our then available repertoire, and that further progress would be dependent upon the attainment of new viewpoints. That was also the year of the last Congresses. Now in the interval, although the world has passed through horrible travail, I think that the type of papers presented at the present program shows that new viewpoints have been attained. Who in Amsterdam in 1938 would ever have thought that we would

t a series of papers discuss

We have not had a paper  
ged the unique properties  
of DDT, the substance that has been known to chemists for very nearly half a century but whose application in this manner was only realized at a comparatively recent date?

I think that we are at the point when new horizons in the control of malaria are opening. And without permitting ourselves to be deluded into great optimism, we may all live to see the point where malaria either vanishes or becomes insignificant as a public health problem.

To me, the most pleasant phase of the Congress has been the opportunity to greet old friends and acquaintances, to renew these pleasant contacts and to make others. In this, I find personally the richest aspect of the meeting.

(The meeting was adjourned at 12.20 p. m.)

ever, with regard to the continuing use of DDT forever, which he seems to think might be an impossible task to impose upon humanity, I would like to suggest that just as the farmer has to protect his crops every year and forever by insecticide sprays, it may well be that we shall have to protect our people by the use of DDT or some better insecticide that may be in the offing. We shall have to look forward to continuous use of this because I don't believe we are going to

antimalarial agent only but probably a general public health measure

Dr L J CIRWATT (Nigeria) I should like Dr Pampana to know what I personally, being a representative of the hyperendemic malaria region, feel about his admirable paper, which condenses so beautifully the present position of the World Health Organization as far as global malaria control is concerned. I should like at the same time to dispel the feeling which was expressed in a very friendly way to me by some of our colleagues, that the hyperendemic malariologists, if I may call them so, have developed a defeatist attitude toward malaria control in hyperendemic areas. I should like to assure my colleagues, and Dr Pampana in particular, that we are only conscious of the very great difficulties of our problem, financial and technical, and the lack of definite knowledge of hyperendemic malaria in Africa. Complete lack of vital statistics in central Africa is another difficulty.

#### CONCLUDING REMARKS OF THE CHAIRMAN AND SECRETARY

Lt Col M K AFRODI (Pakistan), chairman I would like to express the feeling of everyone who has attended this session by pointing out how much we owe to Dr Mark F Boyd, the convener for section V.

Thank you, Colonel

Thank you for your very much appreciated remarks

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